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(54) Title: COMPOSITIONS AND METHODS FOR RAISING HDL CHOLESTEROL LEVELS

(57) Abstract: The present invention relates to LXR agonists and to methods of using such LXR agonists to raise high density lipoprotein (HDL) plasma levels in mammals and to prevent, halt or slow the progression of atherosclerotic cardiovascular diseases and related conditions.

COMPOSITIONS AND METHODS FOR RAISING HDL CHOLESTEROL LEVELS

FIELD OF THE INVENTION

The present invention relates to LXR agonists and to methods of using such LXR agonists to raise high density lipoprotein (HDL) plasma levels in mammals and to prevent, halt or slow the progression of atherosclerotic cardiovascular diseases and related conditions.

BACKGROUND OF THE INVENTION

Hyperlipidemia is a condition which is characterized by an abnormal increase in serum lipids, such as cholesterol, triglycerides and phospholipids. These lipids do not circulate freely in solution in plasma, but are bound to proteins and transported as macromolecular complexes called lipoproteins. There are five classifications of lipoproteins based on their degree of density: chylomicrons; very low density lipoproteins (VLDL); low density lipoproteins (LDL); intermediate density lipoproteins (LDL); and high density lipoproteins (HDL). Such classifications are commonly known to those of skill in the art and are described, for example, in the *Merck Manual,* 16th Ed. 1992 (*see*, for example, pp. 1039-1040) and "Structure and Metabolism of Plasma Lipoproteins" in *Metabolic Basis of Inherited Disease,* 6th Ed. 1989, pp. 1129-1138.

One form of hyperlipidemia is hypercholesterolemia, characterized by the existence of elevated LDL cholesterol levels. The initial treatment for hypercholesterolemia is often to modify the diet to one that is low in fat and cholesterol, coupled with appropriate physical exercise, followed by drug therapy when LDL-lowering goals are not met by diet and exercise alone. LDL is commonly known as the "bad" cholesterol, whereas HDL is the "good" cholesterol. Although it is desirable to lower elevated levels of LDL cholesterol, it is also desirable to increase levels of HDL cholesterol. Generally, it has been found that increased levels of HDL are associated with lower risk for coronary heart disease (CHD). *See*, for example, Gordon, *et al., Am. I. Med.*, *62*:707-7 14 (1977); Stampfer, *et al., N. England J. Med.*, *325*:373-381 (1991); and Kannel, *et al., Ann. Internal Med.*, *90*:85-91

(1979). An example of an HDL raising agent is nicotinic acid, but the quantities needed to achieve HDL raising are associated with undesirable side effects, such as flushing.

Recently, development of therapeutic agents for the treatment of hyperlipidemia and other diseases associated with cholesterol metabolism has been focused on achieving a more complete understanding of the biochemical pathways involved. Most recently, liver X receptors (LXRs) were identified as key components in cholesterol homeostasis. The LXRs were first identified as orphan members of the nuclear receptor superfamily whose ligands and functions were unknown. Two LXR proteins (*i.e.*, α and β) are known to exist in mammals. The expression of LXRα is restricted, with the highest levels being found in the liver and with lower levels being found in the kidneys, intestine, spleen, and adrenals (*see*, Willy, *et al., Genes Dev., 9(9)*:1033-45 (1995)). LXRβ is rather ubiquitous, being found in nearly all tissues examined. Recent studies on the LXRs indicate that they are activated by certain naturally occurring, oxidized derivatives of cholesterol, including 22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol, and 24,25(S)-epoxycholesterol (*see*, Lehmann, *et al., J. Biol. Chem., 272(6)*:3137-3140 (1997)). The expression pattern of LXRs and their oxysterol ligands provided the first hint that these receptors may play a role in cholesterol metabolism (*see*, Janowski, *et al., Nature, 383*:728-731 (1996)).

Cholesterol metabolism in mammals occurs via conversion into steroid hormones or bile acids. The role of LXRs in cholesterol homeostasis was first postulated to involve the pathway of bile acid synthesis, wherein cholesterol 7α-hydroxylase (CYP7α) operates in a rate-limiting manner. Support for this theory was provided when additional experiments found that the CYP7α promoter contained a functional LXR response element that could be activated by RXR/LXR heterodimers in an oxysterol- and retinoid-dependent manner. Confirmation of LXR function as a transcriptional control point in cholesterol metabolism was made using knockout mice, particularly those lacking the oxysterol receptor, LXRα (*see*, Peet, *et al., Cell, 93*:693-704 (1998)).

Mice lacking the receptor LXRα (*e.g.*, knockout or (-/-) mice) lost their ability to respond normally to increases in dietary cholesterol and were unable to tolerate any cholesterol in excess of that synthesized *de novo*. LXRα (-/-) mice did not induce transcription of the gene encoding CYP7α when fed diets containing additional cholesterol. This resulted in an accumulation of large amounts of cholesterol in the livers of LXRα (-/-)

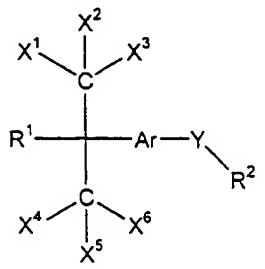
mice, and impaired hepatic function. These results further established the role of LXRα as the essential regulatory component of cholesterol homeostasis. LXRα is also believed to be involved in fatty acid synthesis. Accordingly, regulation of LXRs and, in particular, LXRα could provide treatment for a variety of lipid disorders including obesity and diabetes.

In view of the foregoing, there remains a need in the art for compounds and methods that can be used to regulate LXRs and, in turn, to control the delicate balance of cholesterol metabolism and fatty acid biosynthesis. More particularly, there remains a need in the art for compounds and methods that can be used to increase HDL levels and, thus, to treat disorders associated with bile acid and cholesterol metabolism, including cholesterol gallstones, coronary heart disease, atherosclerosis, lipid storage diseases, obesity, diabetes, *etc.* Quite surprisingly, the present invention fulfills these and other needs by providing such compounds and methods.

SUMMARY OF THE INVENTION

It has now been discovered that ligands which are agonists of LXR and, in particular, LXRα are useful for raising high density lipoprotein (HDL) levels. As such, in one aspect, the present invention provides methods for raising, *i.e.*, increasing, HDL plasma levels in a mammal in need of such treatment, the methods comprising administering to the mammal, *e.g.*, a human, an HDL-raising amount of a LXR agonist and, in particular, a LXRα agonist.

Any compound that activates and, therefore, is an agonist of LXRs can be used in the methods of the present invention. More particularly, any compound that is found to be an agonist of LXR using either *in vitro* or *in vivo* assay procedures, such as those described herein, can be used in the methods of the present invention. In one preferred embodiment, the LXR agonists have the following general formula:



(I)

In Formula I, Ar is an aryl group; R^1 is -OH, -O-(C_1-C_7)alkyl, -OC(O)-(C_1-C_7)alkyl, -CO_2H, -NH_2, -NH(C_1-C_7)alkyl, -N((C_1-C_7)alkyl)$_2$ or -NH-S(O)$_2$-(C_1-C_5)alkyl; R^2 is (C_1-C_7)alkyl, aryl and aryl(C_1-C_7)alkyl; X^1, X^2, X^3, X^4, X^5 and X^6 are each independently -H, (C_1-C_5)alkyl, -F and -Cl, with the proviso that no more than two of X^1 through X^6 are -H or (C_1-C_5)alkyl; and Y is -N(R^{12})S(O)$_m$-, -N(R^{12})S(O)$_m$N(R^{13})-, -N(R^{12})C(O)-, -N(R^{12})C(O)N(R^{13})-, -N(R^{12})C(S)- or -N(R^{12})C(O)O-, wherein R^{12} and R^{13} are each independently hydrogen, (C_1-C_7)alkyl, aryl and aryl(C_1-C_7)alkyl, and optionally when Y is -N(R^{12})S(O)$_m$- or -N(R^{12})S(O)$_m$N(R^{13})-, R^{12} forms a five-, six- or seven-membered ting fused to Ar through covalent attachment to Ar. In the above Y groups, the index "m" is an integer of from 1 to 2.

In another aspect, the present invention provides methods for preventing, halting or slowing the progression of atherosclerotic cardiovascular diseases and related conditions in a mammal in need of such treatment, the methods comprising administering to the mammal an HDL-raising amount of a LXR agonist.

In yet another aspect, the present invention provides methods for preventing, halting or slowing the progression of atherosclerotic cardiovascular diseases and related conditions in a mammal in need of such treatment, the methods comprising administering to the mammal an HDL-raising amount of a LXR agonist in combination with one or more additional active agents, such as bile acid sequestrants, nicotinic acid, fabric acid derivatives, HMG CoA reductase inhibitors, *etc.*

Other features, objects and advantages of the invention and its preferred embodiments will become apparent from the detailed description which follows.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates a schematic diagram of a strategy for the screening of LXRα agonists for use as cholesterol-lowering agents. First, a high throughput screen (HTS) is used to identify compounds that bind to LXRα. Compounds that exhibit binding are next tested for ability to enhance LXRα-mediated transactivation, and for specificity of binding to the LXRα. Those compounds that exhibit favorable activity are then tested for cytotoxicity. Compounds that are nontoxic at the range of expected clinical dosage are then tested for pharmacokinetic (PK) and structure-activity relationship (SAR) activity. Finally,

the lead compounds having the most favorable properties are tested in animal studies, including studies in hypercholesterolemic model systems.

Figure 2 illustrates the results of a radioligand binding assay in which the radiolabeled LXRα agonist T314407 was demonstrated to directly bind to LXRα. Binding of the T314407 to a glutathione-S-transferase alone was negligible.

Figure 3 illustrates the results of a ligand competition assay in which the ability of the LXRα agonist T314407 to compete with other molecules for binding to LXRα was tested. The amount of radiolabeled T314407 that bound to LXRα was competitively inhibited by T314407 itself, by the other agonists T900546 and T901433, and by the known LXRα ligand 24,25-epoxycholesterol.

Figure 4 illustrates that the LXRα agonists T900546 and T314407 competitively inhibit the binding of the radiolabeled oxysterol 24,25-epoxycholesterol to the LXRα receptor.

Figure 5 illustrates the results of a peptide sensor assay in which the LXRα agonists T900546, T314407 and T280404 were shown to induce a conformational change in the LXRα. The oxysterol 24,25-epoxycholesterol also induced a conformational change in the peptide sensor assay.

Figure 6 illustrates the results of a mammalian two-hybrid assay in which the effect of LXRα agonists on transcription mediated by a fusion protein that includes a GAL4 DNA binding domain fused to a SRC-1 polypeptide and a second fusion protein that includes the LXRα ligand binding domain fused to a VP16 activation domain. The amount of expression of a luciferase gene under the control of a GAL4 upstream activation sequence is shown.

Figure 7 illustrates the results of an assay that tested the ability of LXRα agonists to activate LXR-mediated transcription in a cotransfection assay. The amount of expression of a luciferase gene is shown.

Figure 8 presents data which demonstrate that LXRα agonists specifically activate LXR-mediated transcription.

Figure 9 illustrates several analogs of the LXRα agonist T0314407, along with pharmacokinetic data for these analogs.

Figure 10 illustrates the results of an experiment which demonstrates that oral administration of the LXRα agonist T0901317 increases total cholesterol, and also increases the fraction of HDL cholesterol, in mice.

Figure 11 presents data which demonstrate that oral administration of the LXRα agonist T0901317 results in an increase in mouse plasma triglyceride levels.

DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

Definitions

The term "alkyl," by itself or as part of another substituent, refers to, unless otherwise stated, a straight or branched chain, or cyclic hydrocarbon radical, or combination thereof, which may be fully saturated, mono- or polyunsaturated and can include di- and multi-radicals, having the number of carbon atoms designated (*i.e.*, C_1-C_{10} means one to ten carbons). Examples of saturated hydrocarbon radicals include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, cyclohexyl, (cyclohexyl)methyl, cyclopropylmethyl, homologs and isomers of, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like. An unsaturated alkyl group is one having one or more double bonds or triple bonds. Examples of unsaturated alkyl groups include, but are not limited to, vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butynyl, and the higher homologs and isomers. The term "alkyl," unless otherwise noted, is also meant to include those derivatives of alkyl defined in more detail below as "cycloalkyl" and "alkylene." The term "alkylene," by itself or as part of another substituent, refers to a divalent radical derived from an alkane, as exemplified by $-CH_2CH_2CH_2CH_2-$. Typically, an alkyl group will have from 1 to 24 carbon atoms, with those groups having 10 or fewer carbon atoms being preferred in the present invention. A "lower alkyl" or "lower alkylene" refers to a shorter chain alkyl or alkylene group, generally having eight or fewer carbon atoms, preferably four or fewer carbon atoms.

The term "alkoxy," either alone or in combination with other terms, refers to, unless otherwise stated, an alkyl group, as defined above, connected to the remainder of the molecule via an oxygen atom, such as, for example, methoxy, ethoxy, 1 -propoxy, 2-propoxy and the higher homologs and isomers.

The term "heteroalkyl," by itself or in combination with another term, refers to, unless otherwise stated, a stable straight or branched chain, or cyclic hydrocarbon radical,

or combinations thereof, consisting of the stated number of carbon atoms and from one to three heteroatoms selected from the group consisting of O, N, Si and S, and wherein the nitrogen and sulfur atoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) O, N and S may be placed at any interior position of the heteroalkyl group. The heteroatom Si may be placed at any position of the heteroalkyl group, including the position at which the alkyl group is attached to the remainder of the molecule. Examples include, but are not limited to, $-CH_2-CH_2-O-CH_3$, $-CH_2-CH_2-NH-CH_3$, $-CH_2-CH_2-N(CH_3)-CH_3$, $-CH_2-S-CH_2-CH_3$, $-CH_2-CH_2-S(O)-CH_3$, $-CH_2-CH_2-S(O)_2-CH_3$, $-CH=CH-O-CH_3$, $-Si(CH_3)_3$, $-CH_2-CH=N-OCH_3$, and $-CH=CH-N(CH_3)-CH_3$. Up to two heteroatoms may be consecutive, such as, for example, $-CH_2-NH-OCH_3$ and $-CH_2-O-Si(CH_3)_3$. Also included in the term "heteroalkyl" are those radicals described in more detail below as "heteroalkylene" and "heterocycloalkyl." The term "heteroalkylene," by itself or as part of another substituent, refers to a divalent radical derived from heteroalkyl, as exemplified by $-CH_2-CH_2-S-CH_2CH_2-$ and $-CH_2-S-CH_2-CH_2-NH-CH_2-$. For heteroalkylene groups, heteroatoms can also occupy either or both of the chain termini. Still further, for alkylene and heteroalkylene linking groups, as well as all other linking groups described herein, no specific orientation of the linking group is implied.

The terms "cycloalkyl" and "heterocycloalkyl," either by themselves or in combination with other terms, refer to, unless otherwise stated, cyclic versions of "alkyl" and "heteroalkyl," respectively. The terms "cycloalkyl" and "heterocycloalkyl" are intended to include bicyclic, tricyclic and polycyclic versions thereof. Additionally, for heterocycloalkyl, a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyls include, but are not limited to, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, bicyclo[2.2.1]heptyl, bicyclo[2.2.2]octyl, adamantyl, and the like. Examples of heterocycloalkyls include, but are not limited to, 1-(1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholinyl, 3-morpholinyl, 1,4-diazabicyclo[2.2.2]oct-2-yl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1-piperazinyl, 2-piperazinyl, and the like.

The terms "halo" or "halogen," by themselves or as part of another substituent, refer to, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom. Additionally, terms such as "fluoroalkyl," are intended to include monofluoroalkyl and

polyfluoroalkyl while, more generally, "haloalkyl" is intended to include monohaloalkyl and polyhaloalkyl.

The term "aryl," either alone or in combination with other terms (*e.g.*, aryloxy, arylthioxy, arylalkyl), refers to, unless otherwise stated, an aromatic substituent that can be a single ring or multiple rings (up to three rings) that are fused together or linked covalently. The rings may each contain from zero to four heteroatoms selected from N, O, and S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaterized. The aryl groups that contain heteroatoms may be referred to as "heteroaryl" and can be attached to the remainder of the molecule through a carbon atom or a heteroatom. Non-limiting examples of aryl groups include phenyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4isoxazolyl, 5-isoxazolyi, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxalinyl, 5-quinoxalinyl, 3-quinolyl, and 6-quinolyl. Substituents for each of the above noted aryl ring systems are selected from the group of acceptable substituents described below.

The terms "arylalkyl" and "arylheteroalkyl" are intended to include those radicals in which an aryl group is attached to an alkyl group (*e.g.*, benzyl, phenethyl, pyridylmethyl and the like), or a heteroalkyl group (*e.g.*, phenoxymethyl, 2-pyridyloxymethyl, 1-naphthyloxy-3-propyl, and the like). The arylalkyl and arylheteroalkyl groups will typically contain from 1 to 3 aryl moieties attached to the alkyl or heteroalkyl portion by a covalent bond or by fusing the ring to, for example, a cycloalkyl or heterocycloalkyl group. For arylheteroalkyl groups, a heteroatom can occupy the position at which the group is attached to the remainder of the molecule. For example, the term "arylheteroalkyl" is intended to include benzyloxy, 2-phenylethoxy, phenethylamine, and the like.

Each of the above terms (*e.g.*, "alkyl," "heteroalkyl" and "aryl") are meant to include both substituted and unsubstituted forms of the indicated radical. Preferred substituents for each type of radical are provided below.

Substituents for the alkyl and heteroalkyl radicals (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl,

heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) can be a variety of groups selected from the following: -OR', =O, =NR', =N-OR', -NR'R", -SR', -halogen, -SiR'R"R"', -OC(O)R', -CO_2R', -CONR'R", -OC(O)NR'R", -NR"C(O)R', -NR"C(O)NR'R"', -NR"C(O)$_2$R)', -NHC(NH_2)=NH, -NR'C(NH_2)=NH; -NH-C(NH_2)=NR', -S(O)R', -$S(O)_2$R', -$S(O)_2$NR'R", -CN and -NO_2 in a number ranging from zero to (2N+1), where N is the total number of carbon atoms in such radical. Preferably, substituted alkyl groups will have from one to six independently selected substituents, more preferably from one to four independently selected substituents, most preferably from one to three independently selected substituents. In the substituents listed above, R', R" and R"' are each independently selected and are functional groups including, but not limited to, the following: hydrogen, unsubstituted(C_1-C_8)alkyl and heteroalkyl, unsubstituted aryl, aryl substituted with 1-3 halogens, unsubstituted alkyl, alkoxy or thioalkoxy groups, or aryl(C_1-C_4)alkyl groups. When R' and R" are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 5-, 6-, or 7-membered ring. For example, -NR'R" is meant to include 1-pyrrolidinyl and 4-morpholinyl.

Similarly, substituents for the aryl groups are varied and include, but are not limited to the following: -halogen, -OR', -OC(O)R', -NR'R", -SR' -R', -CN, -NO_2, -CO_2R', -CONR'R", -SiR'R"R)", -C(O)R', -OC(O)NR'R", -NR"C(O)R', -NR"C(O)$_2$R', -NR"C(O)NR'R"', -NH-C(NH_2)=NH, -NR'C(NH_2)=NH, -NH-C(NH_2)=NR', -S(O)R', -$S(O)_2$R', S$(O)_2$NR'R", -N_3, -CH(Ph)$_2$, perfluoro(C_1-C_4)alkoxy, and perfluoro(C_1-C_4)alkyl, in a number ranging from zero to the total number of open valences on the aromatic ring system; and wherein R', R" and R"' are independently selected from the following: hydrogen, (C_1-C_8)alkyl, (C_1-C_8)haloalkyl, and (C_1.C_8)heteroalkyl, unsubstituted aryl, (unsubstituted aryl)-(C_1-C_4)alkyl, and (unsubstituted aryl)oxy-(C_1-C_4)alkyl. Preferably, substituted aryl groups will have from one to four independently selected substituents, more preferably from one to three independently selected substituents and, most preferably, from one to two independently selected substituents.

Two of the substituents on adjacent atoms of the aryl ring may optionally be replaced with a substituent of the formula -T-C(O)-$(CH_2)_q$-U-, wherein T and U are independently -NH-, -O-, -CH_2- or a single bond, and q is an integer of from 0 to 2. Alternatively, two of the substituents on adjacent atoms of the aryl ring may optionally be replaced with a substituent of the formula -A-$(CH_2)_r$-B-, wherein A and B are independently

$-CH_2-$, -O-, -NH-, -S-, -S(O)-, $-S(O)_2-$, $-S(O)_2NR'-$ or a single bond, and r is an integer of from 1 to 3. One of the single bonds of the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of the aryl ring may optionally be replaced with a substituent of the formula $-(CH_2)_s-X-(CH_2)_t-$, where s and t are independently integers of from 0 to 3, and X is -O-, NR'-, -S-, -S(O)-, $-S(O)_2-$, or $-S(O)_2NR'-$. The substituent R' in $-NR'-$ and $-S(O)_2NR'-$ is selected from hydrogen or unsubstituted (C_1-C_6)alkyl.

As used herein, the term "heteroatom" is intended to include oxygen (O), nitrogen (N), sulfur (S) and silicon (Si).

The term "pharmaceutically acceptable salt(s)" is meant to include salts of the active compounds which are prepared with relatively nontoxic acids or bases, depending on the particular substituents found on the LXR agonists described herein. When compounds of the present invention contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable base addition salts include, but are not limited to, sodium, potassium, calcium, ammonium, organic amino or magnesium salts, or other similar salts. When compounds of the present invention contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids, such as hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as those derived from relatively nontoxic organic acids, such as acetic, propionic, isobutyric, oxalic, maleic, malonic, benzoic, succinic, suberic, fumaric, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaiic, methanesulfonic, and the like. Also included are salts of amino acids, such as arginate and the like, and salts of organic acids, such as glucuronic or galactunoric acids and the like (*see*, for example, Berge, *et al.*, "Pharmaceutical Salts", *Journal of Pharmaceutical Science*, 66:1-19 (1997)). Certain specific compounds of the present invention contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

The neutral forms of the compounds may be regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but otherwise the salts are equivalent to the parent form of the compound for the purposes of the present invention.

In addition to salt forms, the present invention provides compounds that are in a prodrug form. Prodrugs of the compounds described herein are those compounds that readily undergo chemical changes under physiological conditions to provide a compound of Formula I. Additionally, prodrugs can be converted to the compounds of the present invention by chemical or biochemical methods in an *ex vivo* environment. For example, prodrugs can be slowly converted to the compounds of the present invention when placed in a transdermal patch reservoir with, for example, a suitable enzyme.

Certain compounds of the present invention can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are intended to be encompassed within the scope of the present invention. Certain compounds of the present invention may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present invention and are intended to be within the scope of the present invention.

Certain compounds of the present invention possess asymmetric carbon atoms (optical centers) or double bonds; the racemates, diastereomers, geometric isomers and individual isomers are all intended to be encompassed within the scope of the present invention.

The compounds of the present invention can also contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. For example, the compounds may be radiolabeled with radioactive isotopes, such as tritium (^{3}H), iodine-125 (^{125}I) or carbon- 14 (^{14}C). All isotopic variations of the compounds of the present invention, whether radioactive or not, are intended to be encompassed within the scope of the present invention.

General Overview

The present invention provides methods for preventing or reducing the risk of developing atherosclerosis, the methods comprising the administration of a prophylactically

effective amount or, more particularly, an HDL-raising amount of a LXRα agonist, either alone or in combination with one or more additional pharmaceutically active agents, to a mammal, particularly a human who is at risk of developing atherosclerosis.

LXRα agonists can also be used in methods for treating, halting or slowing the progression of atherosclerotic disease once it has become clinically evident, the methods comprising the administration of a therapeutically effective amount or, more particularly, an HDL-raising amount of a LXRα agonist, either alone or in combination with one or more additional pharmaceutically active agents, to a mammal, particularly a human, who already has atherosclerotic disease.

Atherosclerosis encompasses vascular diseases and conditions that are recognized and understood by physicians practicing in the relevant fields of medicine. Atherosclerotic cardiovascular disease, coronary heart disease (also known as coronary artery disease or ischemic heart disease), cerebrovascular disease and peripheral vessel disease are all clinical manifestations of atherosclerosis and are, therefore, encompassed by the terms "atherosclerosis" and "atherosclerotic disease."

The present invention further provides methods for preventing or reducing the risk of a first or subsequent (where the potential exists for recurrence) atherosclerotic disease event, the methods comprising the administration of a prophylactically effective amount or, more particularly, an HDL-raising amount of a LXRα agonist, either alone or in combination with one or more additional pharmaceutically active agents, to a mammal, particularly a human, who is at risk for having an atherosclerotic disease event. The term "atherosclerotic disease event" as used herein, is intended to encompass coronary heart disease events, cerebrovascular events, and intermittent claudication. Coronary heart disease events are intended to include CHD death, myocardial infarction (*i.e.*, a heart attack), and coronary revascularization procedures. Cerebrovascular events are intended to include ischemic or hemorrhagic stroke (also known as cerebrovascular accidents) and transient ischemic attacks. Intermittent claudication is a clinical manifestation of peripheral vessel disease. It is intended that persons who have previously experienced one or more non-fatal atherosclerotic disease event are those for whom the potential for recurrence of such an event exists.

Persons to be treated with the instant therapy include those at risk of developing atherosclerotic disease and of having an atherosclerotic disease event. Standard

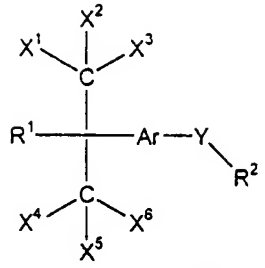
atherosclerotic disease risk factors are known to the average physician practicing in the relevant fields of medicine. Such known risk factors include, but are not limited to, hypertension, smoking, diabetes, low levels of high density lipoprotein cholesterol, high levels of low density lipoprotein cholesterol, and a family history of atherosclerotic cardiovascular disease. Published guidelines for determining those who are at risk of developing atherosclerotic disease can be found in: National Cholesterol Education Program, Second report of the Expert Panel on *Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II),* National Institute of Health, National Heart Lung and Blood Institute, NIH Publication No. 93-3095, September 1993; *abbreviated version:* Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, *Summary of the second report of the national cholesterol education program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II), JAMA,* 1993, *269*:3015-23. People who are identified as having one or more of the above-noted risk factors are intended to be included in the group of people considered at risk for developing atherosclerotic disease. People identified as having one or more of the above-noted risk factors, as well as people who already have atherosclerosis, are intended to be included within the group of people considered to be at risk for having an atherosclerotic disease event.

LXR Agonists

As explained above, the present invention provides methods of raising, *i.e.*, increasing, the plasma level of high density lipoprotein (HDL) in a mammal, the methods comprising administering to the mammal an HDL-raising amount of an LXR agonist. As such, any compound that activates and, therefore, is an agonist of LXRs can be used in the methods of the present invention. More particularly, any compound that is found to be an agonist of LXR using *in vitro* or *in vivo* assay procedures, such as those described herein, can be used in the methods of the present invention.

A. Exemplar LXR Agonists

In one embodiment, the present invention provides LXR agonists that are useful in the methods of the present invention, the LXR agonists having the following general formula:



(I)

In Formula I, "Ar" represents an aryl group. A variety of aryl groups, both substituted and unsubstituted, are useful in the LXR agonists of the present invention. Preferred aryl groups are either monocyclic or fused-bicyclic aromatic rings. Particularly preferred aryl groups include, but are not limited to, benzene, naphthalene, pyridine, quinoline, isoquinoline, pyrrole, furan and thiophene. In a presently preferred embodiment, the aryl group, *i.e.*, Ar, is either a benzene or pyridine ring. In an even more preferred embodiment, the aryl group is a benzene ring.

When the aryl group is a substituted aromatic ring (substituents being in addition to -Y-R^2 and the carbon bearing R^1), the substituents will typically be selected from the following functional groups: -OH, -NH_2, lower alkyl (*e.g.*, methyl, butyl, trifluoromethyl, trifluoroethyl, and the like), lower alkoxy (*e.g.*, methoxy, ethoxy, trifluoromethoxy, butoxy, and the like), -NR'R", -SR', -halogen, -SiR'R"R'", -OC(O)R', -CO_2R', -CONR'R", -C(O)R', -OC(O)NR'R", -NR"C(O)R', -NR"C(O)$_2$R', -NR"C(O)NR'R'", -NHC(NH_2)=NH, -NR'C(NH_2)=NH, -NH-C(NH_2)=NR', -S(O)R', -S(O)$_2$R', -S(O)$_2$NR'R", -CN and -NO_2; wherein R', R" and R'" are each independently selected from the group consisting of hydrogen, (C_1-C_8)alkyl, (C_1-C_8)haloalkyl, (C_1-C_8)heteroalkyl, unsubstituted aryl, (unsubstituted aryl)-(C_1-C_4)alkyl, and (unsubstituted aryl)oxy-(C_1-C_4)alkyl.

The substitutents attached to aryl group can be in any spatial arrangement. For instance, when Ar is a benzene ring, the two groups illustrated in Formula I will preferably be attached to Ar in a 1,3-orientation (meta) or a 1,4-orientation (para). More preferably, the two groups illustrated in Formula I will be attached to a benzene or pyridine ring in a 1,4-orientation (para).

In Formula I, "R'" is a functional group including, but not limited to, the following: -OH, -O-(C_1-C_7)alkyl, -OC(O)-(C_1-C_7)alkyl, -CO_2H, -NH_2,-NH(C_1-C_7)alkyl, -N((C_1-C_7)alkyl)$_2$ or -NH-S(O)$_2$-(C_1-C_5)alkyl. In a preferred embodiment, R^1 is -OH, -CO_2H, -NH_2, -NH(C_1-C_7)alkyl, -N((C_1-C_7)alkyl)$_2$ or -NH-S(O)$_2$-(C_1-C_5)alkyl. In an even more

preferred embodiment, R^1 is -OH. For those embodiments in which R^1 is a dialkylamino group (-N((C_1-C_7)alkyl)$_2$), the alkyl groups can either be the same or different.

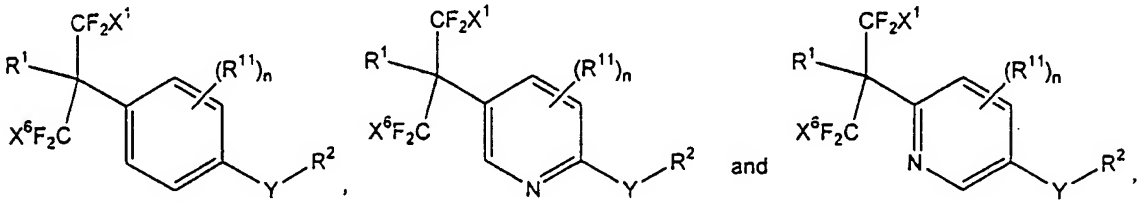
"X^1, X^2, X^3, X^4, X^5 and X^6," in Formula I, are each independently selected and are functional groups including, but not limited to, the following: -H, (C_1-C_5)alkyl, -F and –Cl. X^1 through X^6 are selected such that no more than two of X^1 through X^6 are -H or (C_1-C_5)alkyl. In a preferred embodiment, X^1 through X^6 are selected such that no more than two of X^1 through X^6 are –H, with the remaining being -F. In an even more preferred embodiment, X^1 through X^6 are each -F.

In Formula I, "Y" is a linking group and is selected from -N(R^{12})S(O)$_m$-, -N(R^{12})S(O)$_m$N(R^{13})-, -N(R^{12})C(O)-, -N(R^{12})C(O)N(R^{13})-, -N(R^{12})C(S)- or -N(R^{12})C(O)O-, wherein R^{12} and R^{13} are each independently hydrogen, (C_1-C_7)alkyl, aryl and aryl(C_1-C_7)alkyl, and optionally when Y is -N(R^{12})S(O)$_m$- or -N(R^{12})S(O)$_m$N(R^{13})-, R^{12} forms a five-, six- or seven-membered ring fused to Ar through covalent attachment to Ar. In connection with the linking group, *i.e.*, Y, the index "m" is an integer having a value ranging from 1 to 2. In a preferred embodiment, Y is -N(R^{12})S(O)$_m$-, -N(R^{12})S(O)N(R^{13})- or -N(R^{12})C(O)O-. In an even more preferred embodiment, Y is -N(R^{12})S(O)$_m$-.

As noted above, R^{12} and R^{13} are independently selected from the following functional groups: hydrogen, (C_1-C_7)alkyl, aryl or aryl(C_1-C_7)alkyl, which in the case of the latter two groups, can also be either substituted or unsubstituted. In one preferred embodiment, R^{12} is hydrogen or (C_1-C_4)alkyl, preferably fluoro(C_1-C_4)alkyl. A particularly preferred R^{12} group is 2,2,2-trifluoroethyl. In another preferred embodiment, R^{12} is attached to Ar to form a fused ring system, such as indoline, tetrahydroquinoline or tetrahydroisoquinoline.

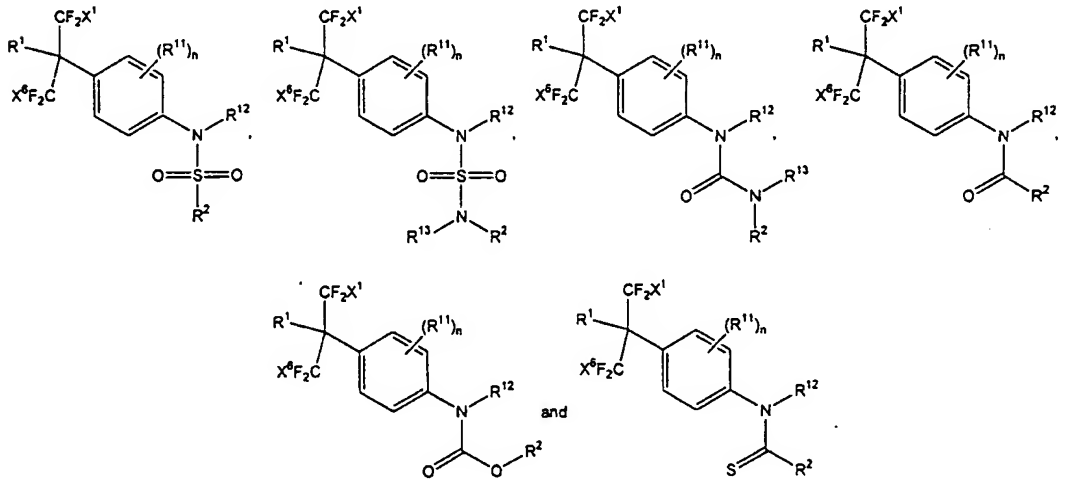
In Formula I, R^2, which is attached to Y, is a functional group including, but not limited to, (C_1-C_7)alkyl, aryl or aryl(C_1-C_7)alkyl. Such R^2 groups can be either substituted or unsubstituted. In preferred embodiments, R^2 is an aryl group. More preferably, R^2 is an aryl group, including, but not limited to, phenyl, thienyl, imidazolyl, oxazolyl and pyridyl. In an even more preferred embodiment, R^2 is phenyl or thienyl (including 2-thienyl and 3-thienyl). Preferred substituted R^2 groups include 3-chlorophenyl, 3-bromophenyl, 3-cyanophenyl, 3-(trifluoromethyl)phenyl, 2-chloro-3-thienyl and 2,5-dichloro-3-thienyl.

Within the scope of Formula I, certain combinations of the above-described functional groups are preferred. For instance, in one preferred embodiment, the LXR agonists of the present invention will be selected from the group consisting of:



In each of the above formulae, the index "n" represents an integer having a value ranging from 0 to 4; and each "R^{11}" is independently selected and is a functional group including, but not limited to, -OH, -NH_2, lower alkyl, lower alkoxy, -NR'R", -SR', -halogen, -SiR'R"R"', -OC(O)R', -C(O)R', -CO_2R', -CONR'R", -OC(O)NR'R", -NR"C(O)R', -NR"C(O)NR'R"', -NR"$C(O)_2$R', -NH-C(NH2)=NH, -NR'$C(NH_2)$=NH, -NH-$C(NH_2)$=NR', -S(O)R', -$S(O)_2$R', -$S(O)_2$NR'R", -CN and -NO_2; wherein R', R" and R"' are each independently selected from the group consisting of hydrogen, (C_1-C_8)alkyl, (C_1-C_8)haloalkyl, (C_1-C_8)heteroalkyl, unsubstituted aryl, (unsubstituted aryl)-(C_1-C_4)alkyl, and (unsubstituted aryl)oxy-(C_1-C_4)alkyl. The remaining groups in the above formulae are as defined above in connection with the LXR agonists set forth in Formula I.

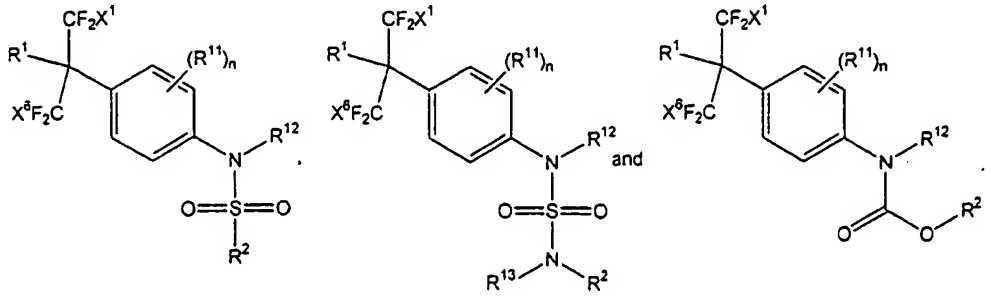
In another preferred embodiment, the LXR agonists of the present invention will be selected from the group consisting of:



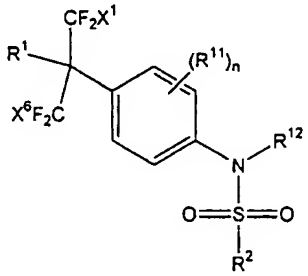
In this group of preferred embodiments, the various groups (*e.g.*, R^1, X^1, X^6, R^2 R^{11}, R^{12} and R^{13}) are as defined above in connection with the LXR agonists of Formula I. Preferably, R^1 is -OH or -NH_2, and X^1 and X^6 are each independently hydrogen or fluorine.

Still further preferred LXR agonists are those embodiments in which R^2 is a substituted aryl group and, more preferably, a substituted phenyl or substituted thienyl group.

In another preferred embodiment, the LXR agonists of the present invention will be selected from the group consisting of:



In yet another preferred embodiment, the LXR agonists of the present invention will have the following formula:



Within this particular group of preferred compounds, certain combinations of the above-described functional groups are particularly preferred. In one such embodiment, R^1 is -OH or -NH^2; X^1 and X^6 are each independently hydrogen or fluorine; R^{12} is fluoro(C_1-C_4)alkyl; and R^2 is aryl (*e.g.*, phenyl). In a second embodiment, R^1 is -OH or -NH_2; X^1 and X^6 are each independently hydrogen or fluorine; R^{12} is hydrogen or (C_1-C_4)alkyl; and R^2 is substituted or unsubstituted thienyl. In a third embodiment, R^1 is -OH or -NH_2; X^1 and X^6 are each independently hydrogen or fluorine, R^{12} is (C_1-C_4)alkyl; and R^2 is phenyl substituted with at least one member selected from the group consisting of -CN, -CF_3, -O-(C_1-C_4)alkyl, -C(O)-(C_1-C_4)alkyl, -C(O)-O(C_1-C_4)alkyl, -C(O)-NH(C_1-C_4)alkyl and -C(O)N((C_1-C_4)alkyl)$_2$. Still further preferred are those embodiments in which R^1 is -OH; and R^2 is phenyl substituted with at least one member selected from -CN, -CF_3 and -O-(C_1-C_4)alkyl.

Further preferred LXR agonists are those in which the compound binds to the ligand binding domain of LXR, more preferably LXRα, with an affinity of at least 10 μM or less and, more preferably, 1 μM or less.

In another embodiment, the present invention provides compounds of Formula I, above, (wherein each of the recited substituents has the meaning provided above) with the proviso that when "-Y-R^2" is -$N(R^{12})S(O)_m$-R^2 or -$N(R^{12})C(O)N(R^{13})$-R^2 and is attached to a position para to the quaternary carbon attached to Ar, and when "R^2" is substituted or unsubstituted phenyl, benzyl or benzoyl, then i) at least one of R^{12} or R^{13} is other than hydrogen or unsubstituted alkyl, or ii) R^2 is substituted with a moiety other than amino, acetamido, di(C_1-C_7)alkylamino, (C_1-C_7)alkylamino, halogen, hydroxy, nitro, or (C_1-C_7)alkyl, or iii) the benzene ring portion of R^2 is substituted with at least three independently selected groups in addition to the Y group.

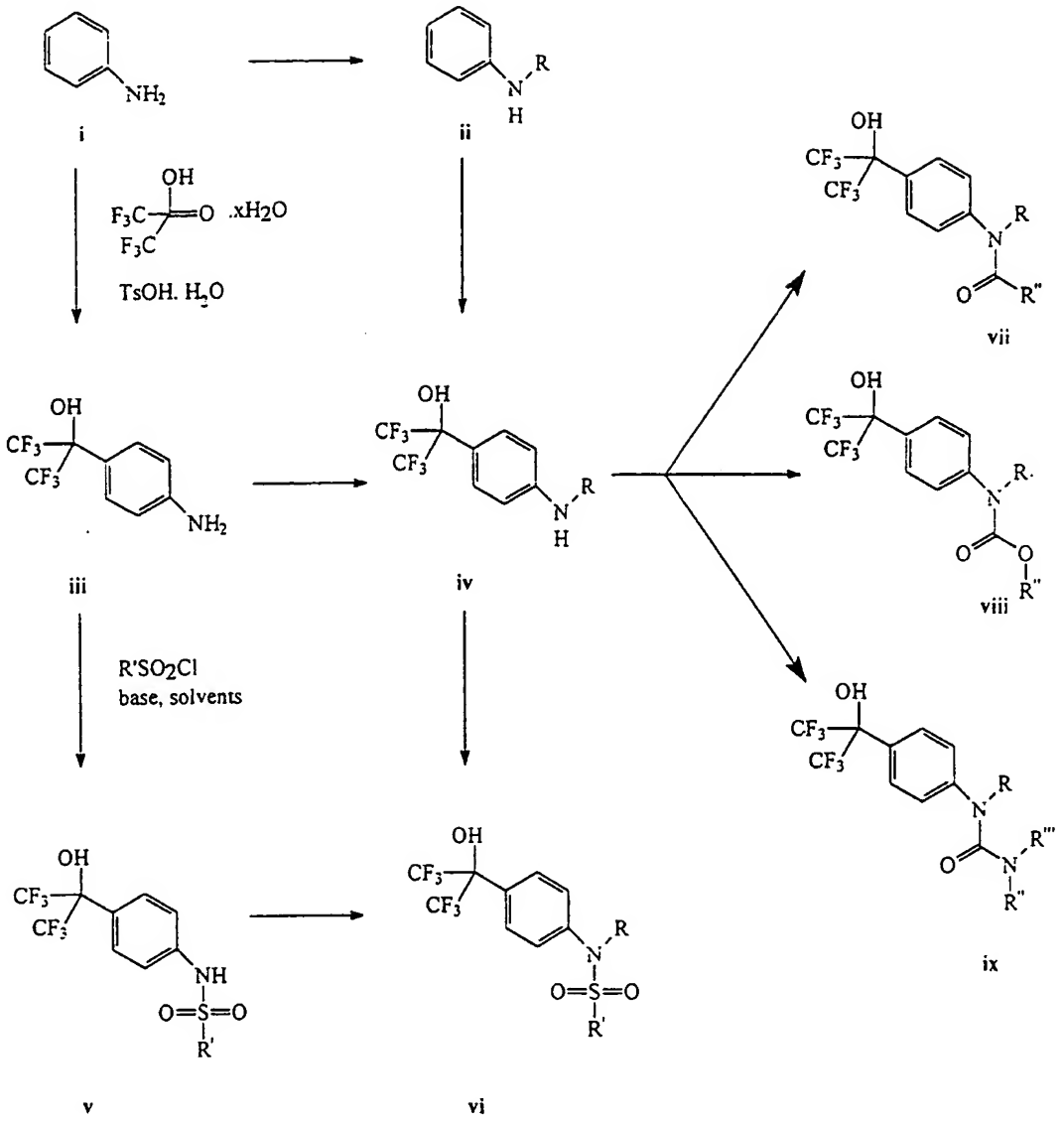
As will be readily apparent to those of skill in the art, some of the compounds of Formula I may exist as stereoisomers, and the invention includes all active stereoisomeric forms of these compounds. In the case of optically active isomers, such compounds may be obtained from corresponding optically active precursors using the procedures described above or by resolving racemic mixtures. The resolution may be carried out using various techniques, such as chromatography, repeated recrystallization of derived asymmetric salts, or derivatization, which techniques are well known to those of ordinary skill in the art.

Moreover, the LXR agonists of the present invention may be labeled in a variety of ways. For example, the compounds may contain radioactive isotopes such as, for example, ^3H (tritium) and ^{14}C (carbon-14). Similarly, the compounds may be advantageously joined, covalently or noncovalently, directly or through a linker molecule, to a wide variety of other compounds, which may provide pro-drugs or function as carriers, labels, adjuvents, coactivators, stabilizers, *etc.* Such labeled and joined compounds are contemplated within the present invention.

B. Snythesis of LXR Agonists

The LXR agonists of Formula I, *supra*, can be prepared using readily available starting materials or known intermediates. Scheme I provides a variety of synthesis avenues for the production of the LXR agonists of the present invention. One of skill in the art will understand that additional methods are also useful.

Scheme I



As illustrated in Scheme I, aniline (**i**, as representative of substituted anilines and other arylamines) can either be alkylated, acylated or arylated (general addition of R group) to form **ii**, or the aromatic ring can derivatized with, for example, hexafluoroacetone to form **iii**. Treatment of **iii** with an appropriate alkylating group, acylating group or arylating group provides **iv**, which can be sulfonylated with, for example, an appropriate sulfonyl halide to form **vi**. Alternatively, the aniline derivative **iii** can be sulfonylated to form v, which can then be alkylated or acylated to form compounds of formula **vi**.

Other LXR agonists of the present invention can be formed by treating the substituted aniline iv (or, alternatively, iii), with reagents suitable for the formation of amides vii, carbamates viii, and ureas ix.

A variety of reagents are useful in the above scheme and can be found in, for example, March, *Advanced Organic Chemistry* 4th Ed., John Wiley & Sons, New York, NY (1992). Preferred reagents and conditions for preparing the LXR agonists are also found in copending U.S. Patent Application Nos. 60/115,292, filed January 8, 1999, and 60/124,525, filed March 15, 1999, the teachings of which are incorporated herein by reference.

C. Screening of Compounds

As explained above, any compound that activates and, therefore, is an agonist of LXR can be used in the methods of the present invention. More particularly, any compound that is found to be an agonist of LXRs and, in particular, LXRα using scientifically sound *in vitro* or *in vivo* assay procedures can be used in the methods of the present invention.

Figure 1 illustrates a schematic diagram of an exemplar strategy that can be used for the identification and screening of LXRα agonists that are useful as cholesterol-lowering agents. First, a high throughput screen (HTS) is used to identify compounds that bind to LXRα. Compounds that exhibit binding are next tested for ability to enhance LXRα-mediated transactivation, and for specificity of binding to the LXRα. Those compounds that exhibit favorable activity are then tested for cytotoxicity. Compounds that are nontoxic at the range of expected clinical dosage are then tested for pharmacokinetic (PK) and structure-activity relationship (SAR) activity. Finally, the lead compounds having the most favorable properties are tested in animal studies, including studies in hypercholesterolemic model systems.

More particularly, compounds can be evaluated *in vitro* for their ability to activate LXR receptor function using biochemical assays (*see*, copending U.S. Patent Application Nos. 08/975,614, filed November 21, 1997, and 09/163,713, filed September 30, 1998), or in cell-based assays, such as that described in Lehnman, *et al.*, *J. Biol. Chem.*, *272(6)*3137-3140 (1997). Alternatively, the compounds and compositions can be evaluated for their ability to increase or decrease gene expression modulated by LXR, using western-blot analysis. Established animal models to evaluate hypocholesterolemic effects of the

compounds are also known in the art. For example, compounds disclosed herein can lower cholesterol levels in hamsters fed a high-cholesterol diet, using a protocol similar to that described in Spady, *et al.*, *J. Clin. Invest.*, *81*:300 (1988); Evans, *et al.*, *J. Lipid Res.*, *35*:1634 (1994), and Lin, *et al.*, *J. Med. Chem.*, *38*:277 (1995). Still further, LXRα animal models (*e.g.*, LXRα (+/-) and (-/-) mice) can be used for evaluation of the present compounds and compositions (*see*, for example, Peet, *et al.*, *Cell*, *93*:693-704 (1998)).

Using the foregoing assays, numerous compounds can be screed for their ability to modulate, *i.e.*, activate, LXRs and, in particular, LXRα. Essentially any chemical compound can be screened as a potential modulator of LXRs, although most often compounds that can be dissolved in aqueous solutions are used. In preferred embodiments, the assays are designed to screen large chemical libraries by automating the assay steps and providing compounds from any convenient source to assay, which are typically run in parallel (*e.g.*, in microtiter formats on microtiter plates in robotic assays). It will be appreciated by those of skill in the art that there are many commercial suppliers of chemical compounds, including Sigma Chemical Co. (St. Louis, MO), Aldrich Chemical Co. (St. Louis, MO), Sigma-Aldrich (St. Louis, MO), Fluka Chemika-Biochemica Analytika (Buchs, Switzerland), and the like.

In one preferred embodiment, high throughput screening methods involve providing a combinatorial library containing a large number of potential therapeutic compounds (*i.e.*, LXR agonists). Such "combinatorial chemical libraries" are then screened in one or more assays, as described herein, to identify those library members (particular chemical species or subclasses) that display a desired characteristic activity, *i.e.*, activate LXRs. The compounds thus identified can serve as conventional "lead compounds" or can themselves be used as potential or actual therapeutics.

A combinatorial chemical library is a collection of diverse chemical compounds generated by either chemical synthesis or biological synthesis, by combining a number of chemical "building blocks," such as reagents. For example, a linear combinatorial chemical library, such as a polypeptide library, is formed by combining a set of chemical building blocks (amino acids) in every possible way for a given compound length (*i.e.*, the number of amino acids in a polypeptide compound). Millions of chemical

compounds can be synthesized through such combinatorial mixing of chemical building blocks.

Preparation and screening of combinatorial chemical libraries is well known to those of skill in the art. Such combinatorial chemical libraries include, but are not limited to, peptide libraries (*see, e.g.*, U.S. Patent No. 5,010,175, Furka, *Int. J. Pept. Prot. Res.*, *37*:487-493 (1991) and Houghton, *et al.*, *Nature*, *354*:84-88 (1991)). Other chemistries for generating chemical diversity libraries can also be used. Such chemistries include, but are not limited to, peptoids (PCT Publication No. WO 91/19735); encoded peptides (PCT Publication WO 93/20242); random bio-oligomers (PCT Publication No. WO 92/00091); benzodiazepines (U.S. Patent No. 5,288,514); diversomers, such as hydantoins, benzodiazepines and dipeptides (Hobbs, *et al.*, *Proc. Nat. Acad. Sci. USA, 90*:6909-6913 (1993)); vinylogous polypeptides (Hagihara, *et al.*, *J. Amer. Chem. Soc. 114*:6568 (1992)); nonpeptidal peptidomimetics with β-D-glucose scaffolding (Hirschmann, *et al.*, *J. Amer. Chem. Soc., 114*:9217-9218 (1992)); analogous organic syntheses of small compound libraries (Chen, *et al.*, *J. Amer. Chem. Soc.*, *116*:2661 (1994)); oligocarbamates (Cho, *et al.*, *Science, 261*:1303 (1993)); and/or peptidyl phosphonates (Campbell, *et al.*, *J. Org. Chem. 59*:658 (1994)); nucleic acid libraries (*see,* Ausubel, Berger and Sambrook, *all supra*); peptide nucleic acid libraries (*see, e.g.,* U.S. Patent No. 5,539,083); antibody libraries (*see, e.g.*, Vaughn, *et al.*, *Nature Biotechnology, 14(3)*:309-314 (1996) and PCT/US96/10287); carbohydrate libraries (*see, e.g.,* Liang, *et al.*, *Science*, *274*:1520-1522 (1996) and U.S. Patent No. 5,593,853); small organic molecule libraries (*see, e.g.,* benzodiazepines, Baum C&E News, Jan. 18, page 33 (1993); isoprenoids (U.S. Patent No. 5,569,588); thiazolidinones and metathiazanones (U.S. Patent No. 5,549,974); pyrrolidines (U.S. Patent Nos. 5,525,735 and 5,519,134); morpholino compounds (U.S. Patent No. 5,506,337); benzodiazepines (U.S. Patent No. 5,288,514); and the like.

Devices for the preparation of combinatorial libraries are commercially available (*see, e.g.*, 357 MPS, 390 MPS, Advanced Chem. Tech, Louisville KY, Symphony, Rainin, Woburn, MA, 433A Applied Biosystems, Foster City, CA, 9050 Plus, Millipore, Bedford, MA). In addition, numerous combinatorial libraries are themselves commercially available (*see, e.g.,* ComGenex, Princeton, N.J., Asinex, Moscow, Ru, Tripos, Inc., St.

Louis, MO, ChemStar, Ltd., Moscow, RU, 3D Pharmaceuticals, Exton, PA, Martek Biosciences, Columbia, MD, *etc.*).

A number of well known robotic systems have also been developed for solution phase chemistries. These systems include automated workstations like the automated synthesis apparatus developed by Takeda Chemical Industries, LTD. (Osaka, Japan) and many robotic systems utilizing robotic arms (Zymate II, Zymark Corporation, Hopkinton, Mass.; Orca, Hewlett-Packard, Palo Alto, Calif.), which mimic the manual synthetic operations performed by a chemist. Any of the above devices are suitable for use with the present invention. The nature and implementation of modifications to these devices (if any) so that they can operate as discussed herein will be apparent to persons skilled in the relevant art. In addition, numerous combinatorial libraries are themselves commercially available (*see, e.g.*, ComGenex, Princeton, N.J., Asinex, Moscow, Ru, Tripos, Inc., St. Louis, MO, ChemStar, Ltd, Moscow, RU, 3D Pharmaceuticals, Exton, PA, Martek Biosciences, Columbia, MD, *etc.*).

Using the *in vitro* assays disclosed herein, compounds can be readily screened for their ability to activate LXRs in a high-throughput format. In such high throughput assays, it is possible to screen up to several thousand different potential LXR agonists in a single day. In particular, each well of a microtiter plate can be used to run a separate assay against a selected potential LXR modulator, or if concentration or incubation time effects are to be observed, every 5-10 wells can test a single LXR modulator. Thus, a single standard microtiter plate can assay about 100 (96) modulators. If 1536 well plates are used, then a single plate can easily assay from about 100- about 1500 different compounds. It is possible to assay many different plates per day; assay screens for up to about 6,000-20,000, and even up to about 100,000-1,000,000 different compounds is possible using the integrated systems of the invention.

High throughput screening systems are commercially available (*see, e.g.*, Zymark Corp., Hopkinton, MA; Air Technical Industries, Mentor, OH; Beckman Instruments, Inc. Fullerton, CA; Precision Systems, Inc., Natick, MA, *etc.*). These systems typically automate entire procedures, including all sample and reagent pipetting, liquid dispensing, timed incubations, and final readings of the microplate in detector(s) appropriate for the assay. These configurable systems provide high throughput and rapid start up as well

as a high degree of flexibility and customization. The manufacturers of such systems provide detailed protocols for various high throughput systems. Thus, for example, Zymark Corp. provides technical bulletins describing screening systems for detecting the modulation of gene transcription, ligand binding, and the like.

LXR Agonist Compositions

The compounds, *i.e.*, the LXR agonists, of this invention can be incorporated into a variety of formulations for therapeutic administration. More particularly, the compounds of the present invention can be formulated into pharmaceutical compositions by combination with appropriate, pharmaceutically acceptable carriers or diluents. Suitable formulations for use in the present invention are found in *Remington's Pharmaceutical Sciences* (Mack Publishing Company, Philadelphia, PA, 17th ed. (1985)), which is incorporated herein by reference. In addition, for a brief review of methods for drug delivery (*see*, Langer, *Science, 249*:1527-1533 (1990), which is incorporated herein by reference).

As used herein, the term "composition" is intended to encompass a product comprising the specified ingredients (*e.g.*, LXR agonist), in specified amounts where amounts are specified, as well as any product that results directly or indirectly from combination of the specified ingredients, in the specified amounts where amounts are specified.

The active LXR agonist compounds of the present invention may be orally administered as a pharmaceutical composition, for example, with an inert diluent, or with an assimilable edible carrier, or they may be enclosed in hard or soft shell capsules, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet. For oral therapeutic administration, which includes sublingual administration, these active compounds may be incorporated with excipients and used in the form of tablets, pills, capsules, ampules, sachets, elixirs, suspensions, syrups, and the like. The active compounds can also be administered intranasally as, for example, liquid drops or spray. Oral administration is preferred. Such compositions and preparations should contain at least 0.1 percent of active compound, *i.e.*, the LXR agonist. The percentage of active compound in these compositions may, of course, be varied and may conveniently be between about 2% to about 60% of the weight of the unit.

Therapeutically effective amounts, prophylactically effective amounts and/or high density lipoprotein-raising amounts of the LXR agonist are suitable for use in the compositions and methods of the present invention. The term "therapeutically effective amount" is intended to mean that amount of a drug or pharmaceutical agent that will elicit the biological or medical response of a tissue, a system, animal or human that is being sought by a clinician, such as a researcher, veterinarian, medical doctor or osteopathic doctor.

The term "prophylactically effective amount" is intended to mean that amount of a drug or pharmaceutical agent that will prevent or reduce the risk of occurrence of a medical condition, such as atherosclerosis or an atherosclerotic disease event.

The term "high density lipoprotein-raising amount" is intended to mean an amount of a drug or pharmaceutical agent that will elevate a subject's plasma HDL level above the level it was at prior to administration of the drug or pharmaceutical agent. Measurement of plasma HDL levels can be performed using any medically acceptable procedures known to those skilled in the medical arts, including assay kits designed for use directly by consumers.

The dosage regimen utilizing a LXR agonist is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal and hepatic function of the patient; and the particular LXR agonist or derivative thereof employed. A consideration of these factors is well within the purview of the ordinarily skilled clinician for the purpose of determining an appropriate HDL-raising amount of the LXR agonist, as well as the therapeutically effective amounts of the LXR agonist needed to prevent, counter, or arrest the progress of the condition.

For example, the compounds of the present invention can be administered at a daily dosage of from about 0.1 milligram to about 100 milligram per kilogram of animal body weight, once a day or given in divided doses two to six times a day, or in sustained release form. For most large mammals, the total daily dosage is from about 1.0 milligram to about 1000 milligrams, and preferably from about 1 milligram to about 50 milligrams. In the case of a 70 kg adult human, the total daily dose will generally be from about 7

milligrams to about 350 milligrams. This dosage regimen may be adjusted to provide the optimal therapeutic response.

The tablets, pills, capsules, and the like may also contain a binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin. When a dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier such as a fatty oil.

Various other materials may be present as coatings or to modify the physical form of the dosage unit. For instance, tablets may be coated with shellac, sugar or both. A syrup or elixir may contain, in addition to the active ingredient, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and a flavoring such as cherry or orange flavor.

These active compounds, *i.e.*, the LXR agonists, may also be administered parenterally. Solutions or suspensions of these active compounds can be prepared in water suitably mixed with a surfactant such as hydroxy-propylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils.

In the above-described methods, the LXR agonist may be administered either alone or in combination with one or more additional active agents. Combination therapy includes administration of a single pharmaceutical dosage formulation which contains a LXR agonist and one or more additional active agents, as well as administration of the LXR

agonist and each active agent in its own separate pharmaceutical dosage formulation. For example, a LXR agonist and an HMG-CoA reductase inhibitor can be administered to the patient together in a single oral dosage composition such as a tablet or capsule, or each agent administered in separate oral dosage formulations. Where separate dosage formulations are used, the LXR agonist and one or more additional active agents can be administered at essentially the same time, *i.e.*, concurrently, or at separately staggered times, *i.e.*, sequentially; combination therapy is understood to include all these regimens.

For example, the LXR agonist may be administered in combination with one or more of the following active agents: an antihyperlipidemic agent; a plasma HDL-raising agent; an antihypercholesterolemic agent, such as a cholesterol biosynthesis inhibitor, for example an HMG-CoA reductase inhibitor, an HMG-CoA synthase inhibitor, a squalene epoxidase inhibitor, or a squalene synthetase inhibitor (also known as squalene synthase inhibitor); an acyl-coenzyme A: cholesterol acyltransferase (ACAT) inhibitor, such as melinamide; probucol; nicotinic acid and the salts thereof and niacinamide; a cholesterol absorption inhibitor such as *beta*-sitosterol; a bile acid sequestrant anion exchange resin, such as cholestyramine, colestipol or a dialkylaminoalkyl derivatives of a cross-linked dextran; an LDL (low density lipoprotein) receptor inducer; fibrates such as clofibrate, fenofibrate, and gemfibrizol; vitamin B_6 (also known as pyridoxine) and the pharmaceutically acceptable salts thereof, such as the HCl salt; vitamin B_{12} (also known as cyanocobalamin); anti-oxidant vitamins, such as vitamin C and E, and beta carotene: a beta-blocker; an angiotensin II antagonist; an angiotensin converting enzyme inhibitor; and a platelet aggregation inhibitor, such as fibrinogen receptor antagonists (*i.e.*, glycoprotein IIb/IIIa fibrinogen receptor antagonists) and aspirin. As noted above, the LXR agonist can be administered in combination with more than one additional active agent, for example, a combination of LXR agonist with an HMG-CoA reductase inhibitor and aspirin, or LXR agonist with an HMG-CoA reductase inhibitor and a *beta* blocker.

The LXR agonist is preferably administered with a cholesterol biosynthesis inhibitor, particularly an HMG-CoA reductase inhibitor. The term HMG-CoA reductase inhibitor is intended to include all pharmaceutically acceptable salt, ester, free acid and lactone forms of compounds which have HMG-CoA reductase inhibitory activity and, therefore, the use of such salts, esters, free acids and lactone forms is included within the

scope of this invention. Compounds which have inhibitory activity for HMG-CoA reductase can be readily identified using assays well-known in the art. For instance, suitable assays are described or disclosed in U.S. Patent No. 4,231,938 and WO 84/02131, the teachings of which are incorporated herein by reference. Examples of suitable HMG-CoA reductase inhibitors include, but are not limited to, lovastatin (MEVACOR®; *see*, U.S. Patent No. 4,231,938); simvastatin (ZOCOR®; *see*, U.S. Patent No. 4,444,784); pravastatin sodium (PRAVACHOL®; *see*, U.S. Patent No. 4,346,227); fluvastatin sodium (LESCOL®; *see*, U.S. Patent No. 5,354,772); atorvastatin calcium (LIPITOR®; *see*, U.S. Patent No. 5,273,995) and rivastatin (also known as cerivastatin; *see*, U.S. Patent No. 5,177,080). The structural formulas of these and additional HMG-CoA reductase inhibitors that can be used in the methods of the present invention are described at page 87 of M. Yalpani, "Cholesterol Lowering Drugs," *Chemistry & Industry*, pp. 85-89 (5 February 1996). In presently preferred embodiments, the HMG-CoA reductase inhibitor is selected from lovastatin and simvastatin.

Dosage information for HMG-CoA reductase inhibitors is well known in the art, since several HMG-CoA reductase inhibitors are marketed in the U.S. In particular, the daily dosage amounts of the HMG-CoA reductase inhibitor may be the same or similar to those amounts which are employed for anti-hypercholesterolemic treatment and which are described in the *Physicians' Desk Reference* (PDR). For example, see the 50th Ed. of the PDR, 1996 (Medical Economics Co); in particular, see at page 216 the heading "Hypolipidemics," sub-heading "HMG-CoA Reductase Inhibitors," and the reference pages cited therein. Preferably, the oral dosage amount of HMG-CoA reductase inhibitor is from about 1 to 200 mg/day and, more preferably, from about 5 to 160 mg/day. However, dosage amounts will vary depending on the potency of the specific HMG-CoA reductase inhibitor used as well as other factors as noted above. An HMG-CoA reductase inhibitor which has sufficiently greater potency may be given in sub-milligram daily dosages.

As examples, the daily dosage amount for simvastatin may be selected from 5 mg, 10 mg, 20 mg, 40 mg, 80 mg and 160 mg; for lovastatin, 10 mg, 20 mg, 40 mg and 80 mg; for fluvastatin sodium, 20 mg, 40 mg and 80 mg; and for pravastatin sodium, 10 mg, 20 mg, and 40 mg. The daily dosage amount for atorvastatin calcium may be in the range of from 1 mg to 160 mg and, more particularly, from 5 mg to 80 mg. Oral administration may

be in single or divided doses of two, three, or four times daily, although a single daily dose of the HMG-CoA reductase inhibitor is preferred.

In accordance with this invention, an HDL-raising amount of a LXR agonist can be used for the preparation of a medicament useful for raising the plasma level of high density lipoprotein in mammals, particularly in humans. Furthermore, a prophylactically effective amount of a LXR agonist can be used for the preparation of a medicament useful for preventing or reducing the risk of developing atherosclerosis, and for preventing or reducing the risk of having a first or subsequent atherosclerotic disease event in mammals, particularly in humans. Also, a therapeutically effective amount of a LXR agonist can be used for the preparation of a medicament useful for treating atherosclerosis in mammals, particularly in humans.

Additionally, in the preparation of the above-described medicaments, the LXR agonist can be admixed together with a therapeutically effective amount of one or more additional active agents selected from the group consisting of: an LDL-lowering agent; an antihyperlipidemic agent; an HDL-raising agent; an HMG-CoA synthase inhibitor; a squalene epoxidase inhibitor; a squalene synthetase inhibitor; an acyl-coenzyme A: cholesterol acyltransferase inhibitor; probucol; nicotinic acid and the salts thereof; niacinamide; a cholesterol absorption inhibitor; a bile acid sequestrant anion exchange resin; a low density lipoprotein receptor inducer; clofibrate, fenofibrate, and gemfibrizol; vitamin B_6 and the pharmaceutically acceptable salts thereof; vitamin B_{12}; an anti-oxidant vitamin; a *beta*-blocker; an angiotensin II antagonist; an angiotensin converting enzyme inhibitor; a platelet aggregation inhibitor; a fibrinogen receptor antagonist; and aspirin.

In particular, the LXR agonist and a therapeutically effective amount of an HMG-CoA reductase inhibitor can be admixed together for the preparation of a medicament useful for the above-described treatments. More particularly, the LXR agonist and a therapeutically effective amount of an HMG-CoA reductase inhibitor selected from the pharmaceutically acceptable lactone, free acid, ester and salt forms of lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin and rivastatin can be admixed together for the preparation of a medicament suitable for oral administration which is useful for the above-described treatments. In a presently preferred embodiment, the HMG-CoA reductase inhibitor used for the medicament preparation is lovastatin or simvastatin.

Without being bound by the present theory, it is thought that the present connection between LXR activation, *e.g.*, by LXR agonists, and HDL levels is mediated by the activation of ABC family members by LXR. Specifically, it has been discovered that LXR agonists can induce the expression of ABC family members, in particular ABC family members involved in sterol transport. In particular, LXR activation leads to a dramatic increase in the transcription of ABC family members. This increased ABC activity, in turn, causes an increase in the transport of sterols, *e.g.*, cholesterol, and other lipids across the membranes of cells, thereby leading to an overall increase in HDL levels.

EXAMPLES

The invention will be described in greater detail by way of specific examples. The following examples are offered for illustrative purposes, and are not intended to limit the invention in any manner. Those of skill in the art will readily recognize a variety of noncritical parameters which can be changed or modified to yield essentially the same results.

Example 1

Identification and Characterization of LXRα Agonists

In this example, several compounds are tested for their ability to act as LXRα agonists (*see*, Figure 1). First, the compounds were tested for ability to bind to LXRα. LXRα agonists were then identified in both a cell-based high throughput screen and a peptide sensor LXR assay.

LXRα Binding Ability

First, the ability of putative agonists to bind to LXRα was tested. A fusion protein in which the LXRα is fused to glutathione-S-transferase (GST) was produced. The ability of radiolabeled putative agonist compounds to bind to the fusion protein was then tested.

Results of a radioligand binding assay are shown in Figure 2. The assay demonstrates that the compound T314407 directly binds to the LXRα fusion protein, and does not bind appreciably to GST alone.

The compound T314407 was also tested for ability to compete with other ligands for binding to LXRα. The percentage of radiolabeled T314407 that bound to LXRα

in the presence of varying concentrations of unlabeled T314407, T900546, T901,433, or 24,25-epoxycholesterol was determined. Results, which are shown in Figure 3, demonstrate that T314407 competitively binds to LXRα with a K_i of 0.2 μM.

In a further experiment, the ability of T314407 and T900546 to compete with oxysterol for binding to LXRα was tested. The amount of radiolabeled oxysterol ^{3}H-24,25-epoxycholesterol was compared in the presence of varying concentrations of unlabeled 24,25-epoxycholesterol or the putative LXR agonists T900546 and T314407. As shown in Figure 4, both T900546 and T314407 were able to compete with the oxysterol for binding to LXRα.

Ligand-mediated Conformational Change of LXRα

In this Example, a peptide sensor assay was used to demonstrate the ability of a putative LXRα agonist to induce a conformational change in LXRα. A ligand-induced conformational change is believed to be a common property of nuclear hormone receptors such as LXRα. The peptide sensor assay, which was carried out as described in PCT patent application PCT/US98/24969 (Publ. No. WO 99/27365), is an *in vitro* assay.

As shown in Figure 5, the addition of increasing amounts of the LXRα agonists T900546, T314407, and T280404 resulted in a conformational change, as evidenced by the increase in fluorescence. The oxysterol 24,25-epoxycholesterol also resulted in an increase in fluorescence.

Example 2

Effect of LXR Agonists on LXRα-mediated Transcription

The ability of LXR agonists to stimulate transcription of LXRα-mediated transcription was examined in the experiments described in this Example. First, the effect of the agonist T314407 on the recruitment of the coactivator SRC-1 to LXRα was examined in a mammalian two-hybrid assay. The ability of T314407 to activate LXR-mediated transcription was then tested in a reporter gene cotransfection assay was then tested. Finally, the effect of various LXR agonists on transcription was shown to be LXR-specific.

Briefly, for the cell-based assay, a DNA binding domain of the nonreceptor transcription factor GAL4 was fused to the putative ligand binding domain of LXRα. The resultant construct was introduced into 293 cells, together with a luciferase reporter construct under the control of a GAL4 upstream activation sequence (UAS). The transfected cells

were then treated with the compounds and luciferase activity was measured. Individual compounds were evaluated relative to a control (no additional compound) and the EC_{50} was determined as the concentration necessary to produce 50% of the maximal luciferase activity.

LXRα Agonists Enhance Recruitment of SRC-1 Coactivator

In the first of these experiments, a fusion polypeptide in which the DNA binding domain of the GAL4 transcription factor was fused to the SRC-1 coactivator. A plasmid expressing the GAL4 DNA-binding domain-SRC-1 fusion protein and a plasmid expressing second fusion protein in which an LXRα ligand-binding domain was fused to VP-16 were used. HepG2 cells were transiently transfected with pG5 (a luciferase reporter coding sequence under the control of five GAL4 binding sites) luciferase reporter plasmid (0.25 μg/1.5 x 10^5 cells) and the plasmids expressing the two fusion proteins. Luciferase reporter activity was measured after treating cells with or without the LXR agonists (concentrations as indicated). Relative luciferase activity is a ratio of luciferase activity (normalized by β-galactosidase activity) between treated and untreated cells.

The putative LXR agonists T314407 and T280404 markedly stimulated the recruitment of the SRC-1 coactivator to LXRα, as shown in Figure 6. The oxysterol 24,25-epoxycholesterol also stimulated recruitment, while the DMSO control indicated no enhancement of recruitment.

LXRα Agonists Activate LXR-mediated Transcription

The ability of the LXRα agonists T280404 and T314407 to activate LXR-mediated transcription was then tested. The effect of the putative agonist compounds on LXR-mediated transcription of the luciferase reporter gene is shown in Figure 7. Both T280404 and T314407 activated LXR-mediated transcription.

The LXRα Agonists Specifically Activate LXR-mediated Transcription

In this experiment, the effect of LXRα agonists on transcription activation by LXRα and other nuclear receptors was examined. Plasmids that encode fusion proteins of the ligand binding domains of LXRα, LXRβ, CPF, HNF4, FXR, and RXR were constructed by introducing the respective coding regions into the plasmid pM3 (Clontech, Inc.), which includes a GAL4 DNA binding domain. The resulting plasmids were individually co-

transfected into the cells along with a reporter plasmid that contained a luciferase reporter gene under the control of a GAL4 upstream activation sequence. Relative luciferase activity was determined in the presence or absence of the putative LXRα agonists T170400, T280404, T314393, T314407, T513892, T210943, and T588142, as well as the known LXRα modulator 24,25-epoxycholesterol.

As shown in Figure 8, each of the putative LXRα agonists strongly activated LXR-mediated transcription. A lesser amount of activation was observed for LXRβ-mediated transcription. The LXRα agonists did not activate transcription mediated by CPF, HNF4, FXR, or RXR.

The highest amount of activation was observed for the compound T314407, which activated LXRα-mediated transcription with an EC_{50} of 0.2 μM (EC_{50} is defined as the amount of compound necessary to product 50% of the maximal luciferase activity). This compound directly binds to LXRα, as evidenced by the competition assay described above, with a K_i of 0.2 μm. Moreover, T314407 is not cytotoxic, having an EC_{50} of greater than 50μm. T314407 was also demonstrated to transactivate expression of the human cholesterol 7α-hydrolase (CYP7A) gene, which is a rate limiting enzyme in bile acid synthesis, which is a major pathway for cholesterol catabolism. Thus, T314407 and other LXRα agonists are useful for lowering cholesterol levels and for treating other lipid disorders.

Analogs of T0314407 were tested for properties that are desirable for a cholesterol-lowering, HDL-cholesterol increasing pharmaceutical agent. The structures of several analogs, and the corresponding pharmacokinetic data for the compounds are shown in Figure 9. The activity of the compounds in peptide sensor (FP), luciferase expression, cytotoxicity, and plasma concentration are shown. One of these analogs, T0901317, was chosen for further study *in vivo*.

Example 3

Oral Administration of LXR Agonist Increases HDL Cholesterol Levels In vivo

This Example describes experiments to determine the effect of oral administration of the LXR agonist T0901317 on plasma triglyceride levels and HDL cholesterol levels. The study was conducted over two weeks, with twenty mice (*C57BL/6*) for each timepoint (10 males and 10 females). T090137 was administered to the mice once a

day at doses of 5 or 50 mg/kg body weight. At 7 and 14 days, blood of the mice was analyzed for plasma lipid concentration and for hepatic gene expression.

Oral administration of 5 mg/kg and 50 mg/kg T090137 resulted in an increase in total plasma cholesterol in both male and female mice (Figure 10, left panel). HDL cholesterol levels were also increased by administration of the LXR agonist (Figure 10, right panel). The amount of the increase in total plasma cholesterol and in HDL cholesterol was dependent on the amount of T090137 administered in each dose, but 14 days of administration did not result in appreciable difference in effect compared to a 7 day administration regime. Oral administration of T0901317 also resulted in an increase in plasma triglyceride levels (Figure 11). These results were not changed significantly by feeding the mice a high cholesterol diet.

These results demonstrate that oral administration of the LXR agonist T0901317 can increase the HDL-cholesterol fraction in mice. HDL is directly protective against atherogenesis, so these results indicate that administration of an LXR agonist can protect against atherosclerosis.

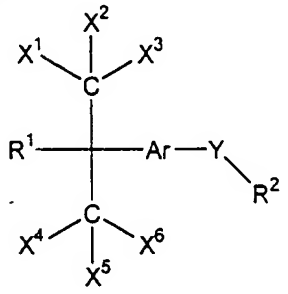
It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference for all purposes.

WHAT IS CLAIMED IS:

1. A method for raising the plasma level of high density lipoprotein (HDL) in a mammal, said method comprising: administering to said mammal an HDL-raising amount of an LXR agonist.

2. The method in accordance with claim 1, wherein said LXR agonist is an LXR-α agonist.

3. The method in accordance with claim 1, wherein said LXR agonist has the following general formula:



or a pharmaceutically acceptable salt thereof, wherein:

Ar is an aryl group;

R^1 is a member selected from the group consisting of -OH, -O-(C_1-C_7)alkyl, -OC(O)-(C_1-C_7)alkyl, -CO_2H, -NH_2, -NH(C_1-C_7)alkyl, -N((C_1-C_7)alkyl)$_2$ and -NH-S(O)$_2$-(C_1-C_5)alkyl;

R^2 is a member selected from the group consisting of (C_1-C_7)alkyl, aryl and aryl(C_1-C_7)alkyl;

X^1, X^2, X^3, X^4, X^5 and X^6 are each independently selected from the group consisting of -H, (C_1-C_5)alkyl, -F and -Cl, with the proviso that no more than two of X^1 through X^6 are -H or (C_1-C_5)alkyl; and

Y is a member selected from the group consisting of -N(R^{12})S(O)$_m$-, -N(R^{12})S(O)$_m$N(R^{13})-, -N(R^{12})C(O)-, -N(R^{12})C(O)N(R^{13})-, -N(R^{12})C(S)- and -N(R^{12})C(O)O-, wherein R^{12} and R^{13} are each independently selected from the group consisting of hydrogen, (C_1-C_7)alkyl, aryl and aryl(C_1-C_7)alkyl, and optionally when Y is -

$N(R^{12})S(O)_m$- or -$N(R^{12})S(O)_mN(R^{13})$-, R^{12} forms a five-, six- or seven-membered ring fused to Ar through covalent attachment to Ar; and m is an interger having a value ranging from 1 to 2.

4. The method in accordance with claim 3, wherein said aryl group is a member selected from the group consisting of benzene, naphthalene, pyridine, quinoline, isoquinoline, pyrrole, furan and thiophene.

5. The method in accordance with claim 3, wherein said aryl group is benzene.

6. The method in accordance with claim 3, wherein R^1 is a member selected from the group consisting of -OH, -CO_2H, -NH_2, -NH(C_1-C_7)alkyl, -N((C_1-C_7)alkyl)$_2$ and -NH-S(O)$_2$-(C_1-C_5)alkyl.

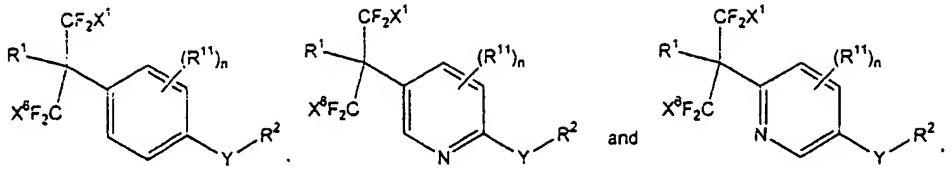
7. The method in accordance with claim 3, wherein R^2 is an aryl group.

8. The method in accordance with claim 7, wherein said aryl group is phenyl.

9. The method in accordance with claim 8, wherein said phenyl is substituted with at least one substitutent at the ortho or meta position relative to the point of attachment to Y.

10. The method in accordance with claim 3, wherein no more than two of X^1 through X^6 are -H or (C_1-C_5)alkyl.

11. The method in accordance with claim 3, wherein said LXR agonist is a member selected from the group consisting of

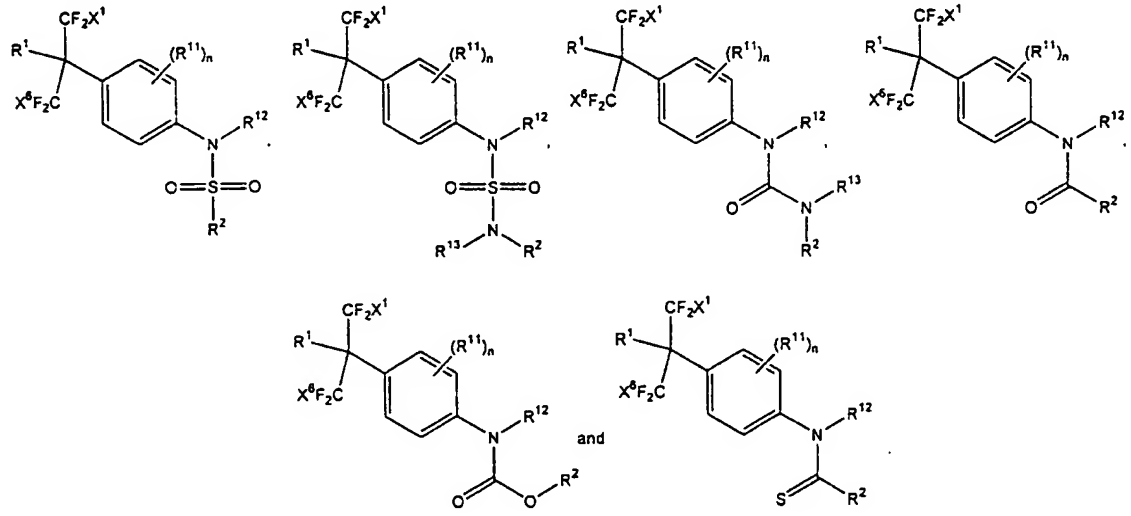


wherein:

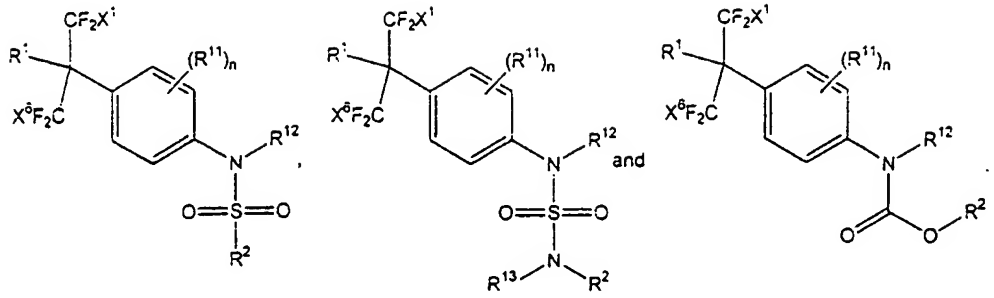
n is an interger having a value ranging from 0 to 4; and

each R^{11} is independently selected from the group consisting of -OH, -NH_2, lower alkyl, lower alkoxy, -NR'R", -SR', -halogen, -SiR'R"R"', -OC(O)R', -C(O)R', -CO_2R', -CONR'R", -OC(O)NR'R", -NR"C(O)R', -NR"C(O)NR'R"', -NR"$C(O)_2$R', -NH-C(NH2)=NH, -NR'$C(NH_2)$=NH, -NH-$C(NH_2)$=NR', -S(O)R', -$S(O)_2$R', -$S(O)_2$NR'R", -CN and -NO_2; wherein R', R" and R"' are each independently selected from the group consisting of hydrogen, (C_1-C_8)alkyl, (C_1-C_8)haloalkyl, (C_1-C_8)heteroalkyl, unsubstituted aryl, (unsubstituted aryl)-(C_1-C_4)alkyl, and (unsubstituted aryl)oxy-(C_1-C_4)alkyl.

12. The method in accordance with claim 11, wherein said LXR agonist is a member selected from the group consisting of



13. The method in accordance with claim 3, wherein wherein said LXR agonist is a member selected from the group consisting of

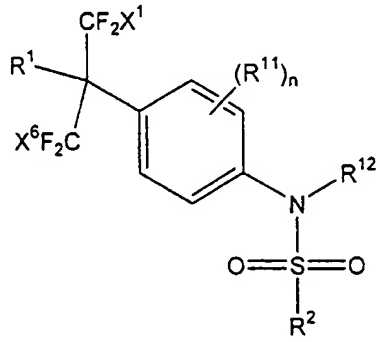


3

1 14. The method in accordance with claim 3, wherein X^1 through X^6 are
2 each -F.

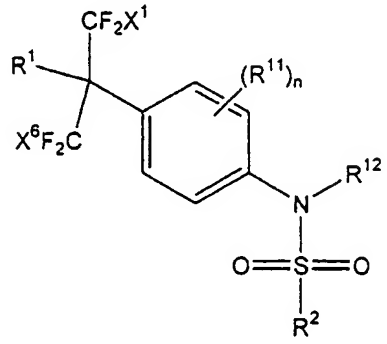
1 15. The method in accordance with claim 3, wherein X^1 through X^6 are each
2 -F; R^1 is a member selected from the group consisting of -OH, $-CO_2H$, $-NH_2$, $-NH(C_1$-
3 $C_7)$alkyl, $-N((C_1-C_7)alkyl)_2$ and $-NH-S(O)_2-(C_1-C_5)$alkyl; Y is a member selected from the
4 group consisting of $-N(R^{12})S(O)_m-$, $-N(R^{12})C(O)-$, $-N(R^{12})C(O)N(R^{13})-$; and R^2 is aryl.

1 16. The method in accordance with claim 13, wherein said LXR agonist has
2 the following formula:



3 wherein:
4 R^1 is a member selected from the group consisting of -OH and $-NH^2$; X^1
5 and X^6 are each independently selected from the group consisting of hydrogen and fluorine;
6 R^{12} is fluoro(C_1-C_4)alkyl; and R^2 is aryl.

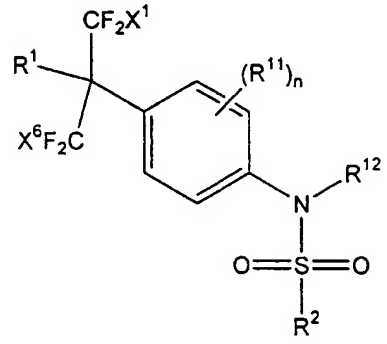
17. The method in accordance with claim 13, wherein said LXR agonist has the following formula:



wherein:

R^1 is a member selected from the group consisting of -OH and -NH^2; X^1 and X^6 are each independently selected from the group consisting of hydrogen and fluorine; R^{12} is fluoro(C_1-C_4)alkyl; and R^2 is a substituted or unsubstituted thienyl.

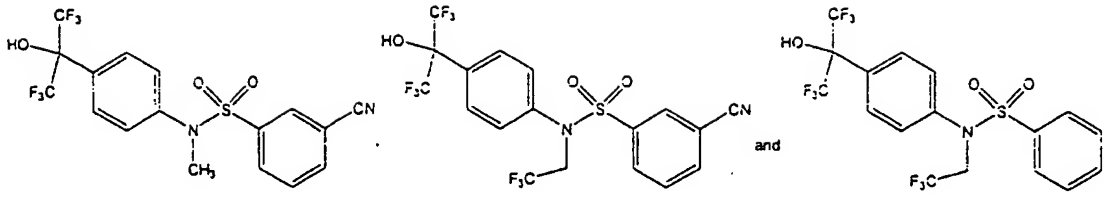
18. The method in accordance with claim 13, wherein said LXR agonist has the following formula:



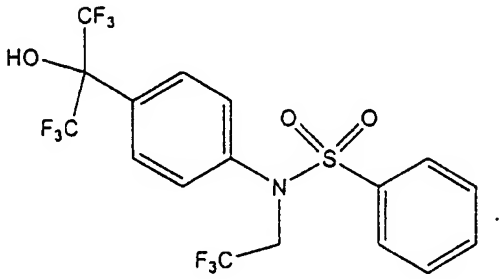
wherein:

R1 is a member selected from the group consisting of -OH and -NH^2; X^1 and X^6 are each independently selected from the group consisting of hydrogen and fluorine; R^{12} is fluoro(C_1-C_4)alkyl; and R^2 is a substituted or unsubstituted phenyl.

19. The method in accordance with claim 18, wherein said LXR agonist is a compound selected from the group consisting of:



20. The method in accordance with claim 19, wherein said LXR agonist has the following structure:



21. The method in accordance with claim 1, wherein said mammal is a human.

22. The method in accordance with claim 21, wherein said human is at risk of developing atherosclerosis or having an atherosclerotic-associated disease.

23. The method in accordance with claim 21, wherein said human has atherosclerosis.

24. The method in accordance with claim 1, further comprising administering to said mammal an additional active agent selected from the group consisting of an antihyperlipidemic agent; a plasma HDL-raising agent; an antihypercholesterolemic agent; a cholesterol biosynthesis inhibitor; an acyl-coenzyme A: cholesterol acyltransferase inhibitor; probucol; nicotinic acid and the salts thereof; niacinamide; a cholesterol absorption inhibitor; a bile acid sequestrant anion exchange resin; a low density lipoprotein receptor inducer; Clofibrate, fenofibrate, and gemfibrizol; vitamin B_6 and the pharmaceutically acceptable salts thereof; vitamin B_{12}; an anti-oxidant vitamin; a *beta*-blocker; an angiotensin

II antagonist; an angiotensin converting enzyme inhibitor; a platelet aggregation inhibitor; a fibrinogen receptor antagonist; and aspirin.

25. The method in accordance with claim 24, wherein said additional active agent is a cholesterol biosynthesis inhibitor.

26. The method in accordance with claim 25, wherein said cholesterol biosynthesis inhibitor is an HMG-CoA reductase inhibitor.

27. The method in accordance with claim , wherein said HMG-CoA reductase inhibitor is selected from the group consisting of lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin and rivastatin.

28. The method in accordance with claim 1, wherein said LXR agonist has an EC_{50} of 10 μM or less in a transcription assay.

29. The method in accordance with claim 1, wherein said LXR agonist has an EC_{50} of 1.0 μM or less in a transcription assay.

30. The method in accordance with claim 1, wherein said LXR agonist has a Ki of 10 μM or less in a competitive binding assay with 24,25-epoxycholesterol.

31. The method in accordance with claim 1, wherein said LXR agonist has a Ki of 1.0 μM or less in a competitive binding assay with 24,25-epoxycholesterol.

32. The method in accordance with claim 1, wherein said LXR agonist has a Ki of 0.1 μM or less in a competitive binding assay with 24,25-epoxycholesterol.

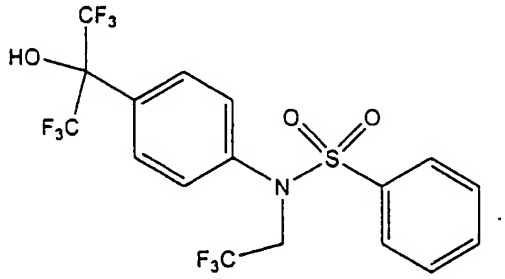
33. The method in accordance with claim 1, wherein said LXR agonist has an $EC_{50} \geq 30$ μM in a cytotoxicity assay.

34. A method for treating atherosclerosis in a mammal, said method comprising: administering to said mammal an effective amount of an LXR agonist.

35. The method in accordance with claim 34, wherein said LXR agonist is an LXRα agonist.

36. The method in accordance with claim 34, wherein said LXR agonist is an LXR agonist of claim 3.

37. The method in accordance with claim 34, wherein said LXR agonist has the following structure:



38. The method in accordance with claim 34, wherein said mammal is a human.

39. The method in accordance with claim 34, further comprising administering to said mammal an additional active agent selected from the group consisting of an antihyperlipidemic agent; a plasma HDL-raising agent; an antihypercholesterolemic agent; a cholesterol biosynthesis inhibitor; an acyl-coenzyme A: cholesterol acyltransferase inhibitor; probucol; nicotinic acid and the salts thereof; niacinamide; a cholesterol absorption inhibitor; a bile acid sequestrant anion exchange resin; a low density lipoprotein receptor inducer; Clofibrate, fenofibrate, and gemfibrizol; vitamin B_6 and the pharmaceutically acceptable salts thereof; vitamin B_{12}; an anti-oxidant vitamin; a beta-blocker; an angiotensin II antagonist; an angiotensin converting enzyme inhibitor; a platelet aggregation inhibitor; a fibrinogen receptor antagonist; and aspirin.

40. A method for reducing the risk of developing atherosclerosis or an atherosclerotic-associated disease in a mammal, said method comprising: administering to said mammal an prophylactically effective amount of an LXR agonist.

41. The method in accordance with claim **40**, wherein said LXR agonist is an LXR agonist of claim **3**.

42. A post-myocardial infarction therapy, said therapy comprising: administering to a human who has suffered a mycocardial infacrtion an HDL-raising amount of an LXR agonist.

43. The method in accordance with claim **42**, wherein said LXR agonist is an LXR agonist of claim **3**.

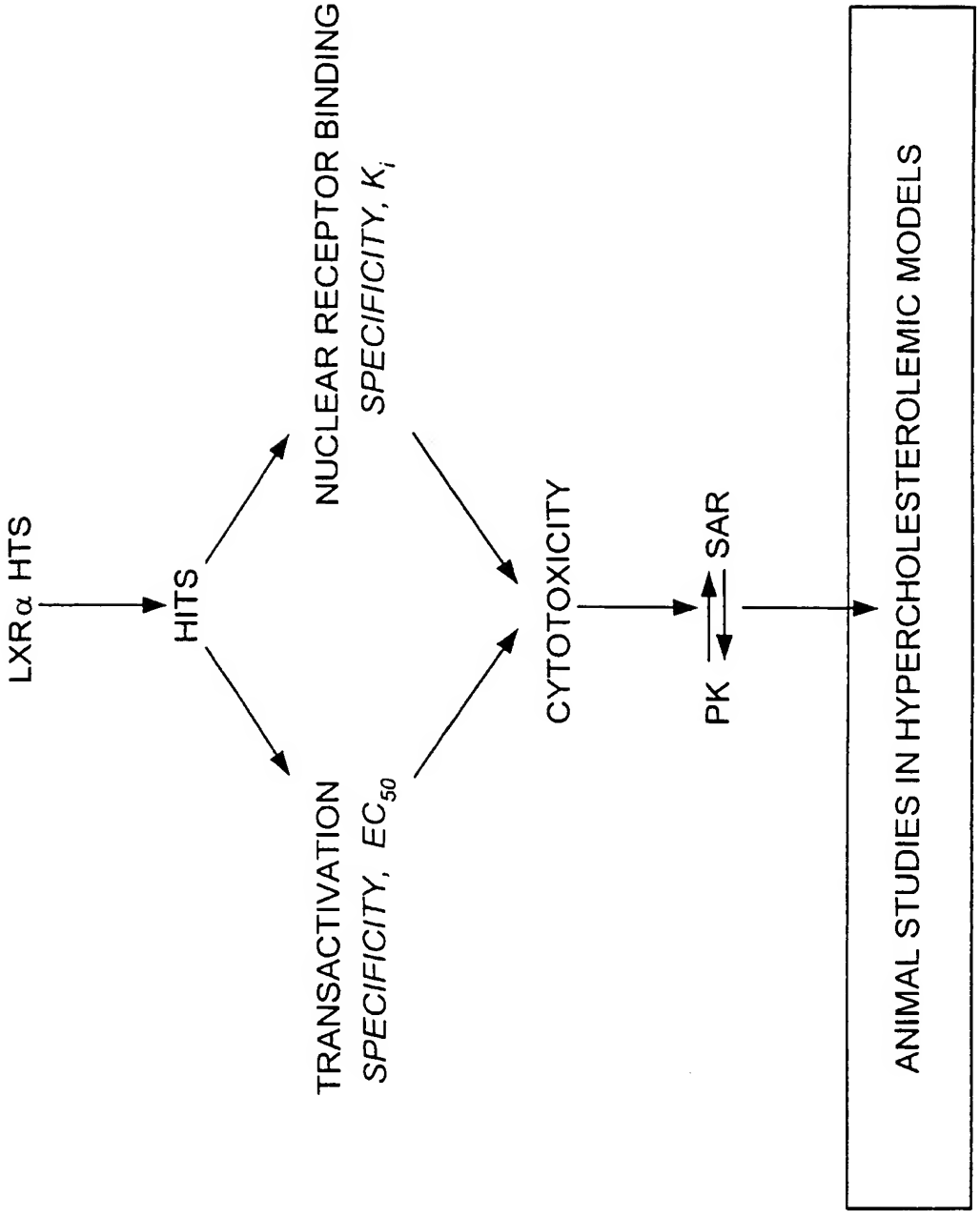

LXRα HTS
HITS
NUCLEAR RECEPTOR BINDING
SPECIFICITY, K_i
TRANSACTIVATION
SPECIFICITY, EC_{50}
CYTOTOXICITY
PK
SAR
ANIMAL STUDIES IN HYPERCHOLESTEROLEMIC MODELS

FIG. 1.

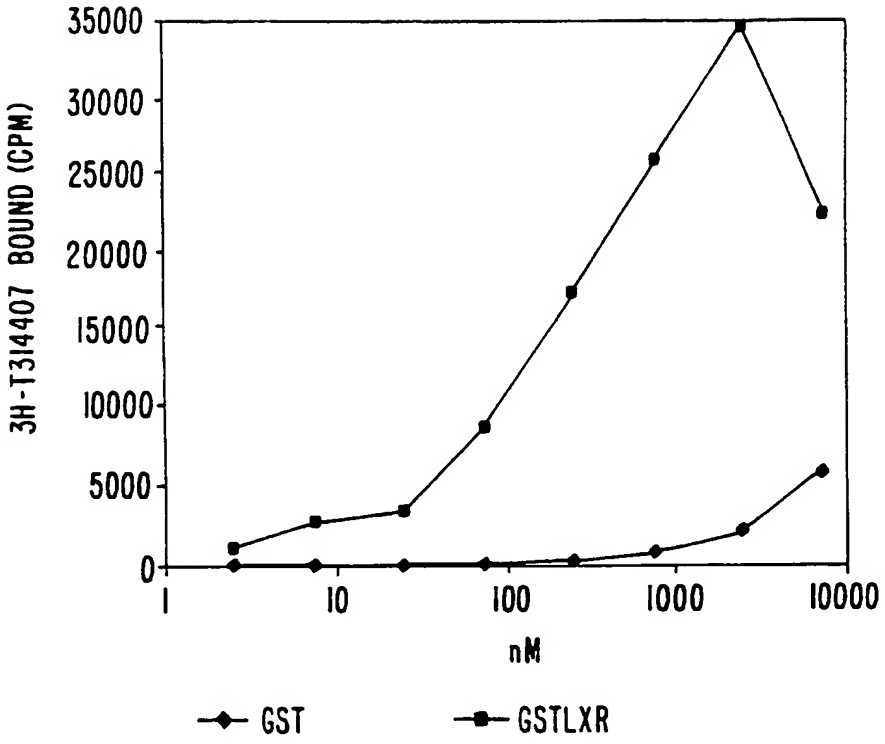

35000
30000
25000
20000
15000
10000
5000
0
3H-T314407 BOUND (CPM)
1
10
100
1000
10000
nM
GST
GSTLXR

FIG. 2.

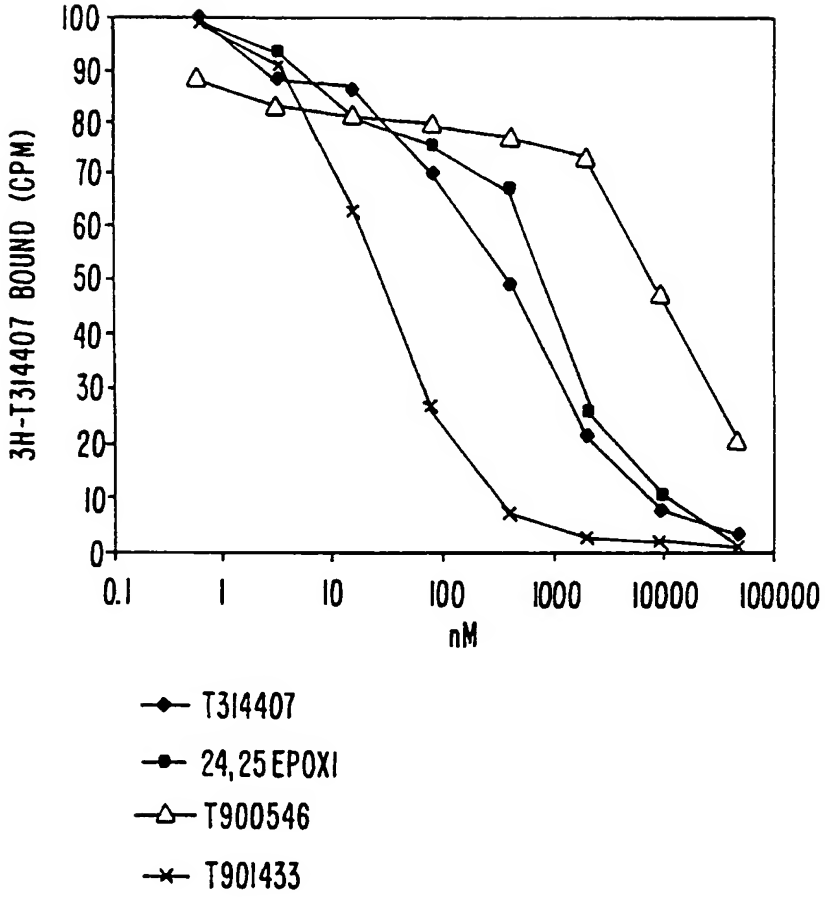

3H-T314407 BOUND (CPM)
100
90
80
70
60
50
40
30
20
10
0
0.1
1
10
100
1000
10000
100000
nM
T314407
24,25EPOXI
T900546
T901433

FIG. 3.

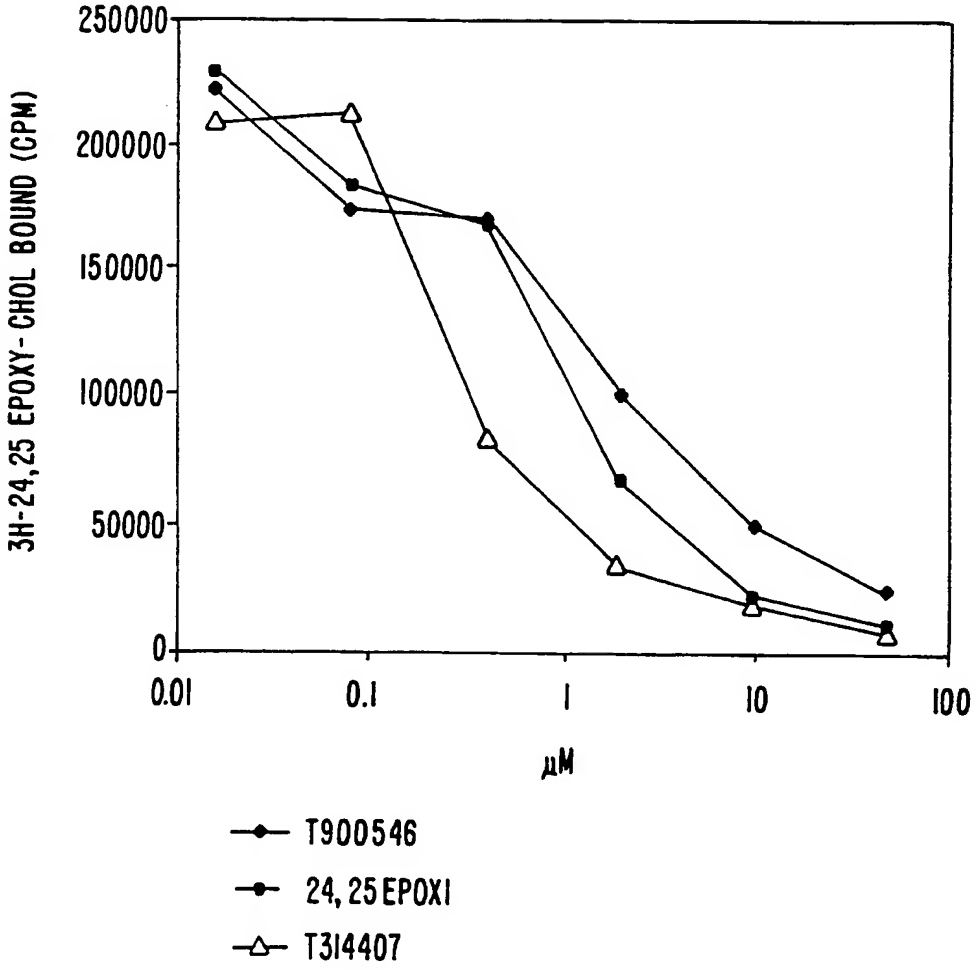

250000
200000
150000
100000
50000
0
3H-24,25 EPOXY-CHOL BOUND (CPM)
0.01
0.1
1
10
100
μM
T900546
24,25EPOXI
T314407

FIG. 4.

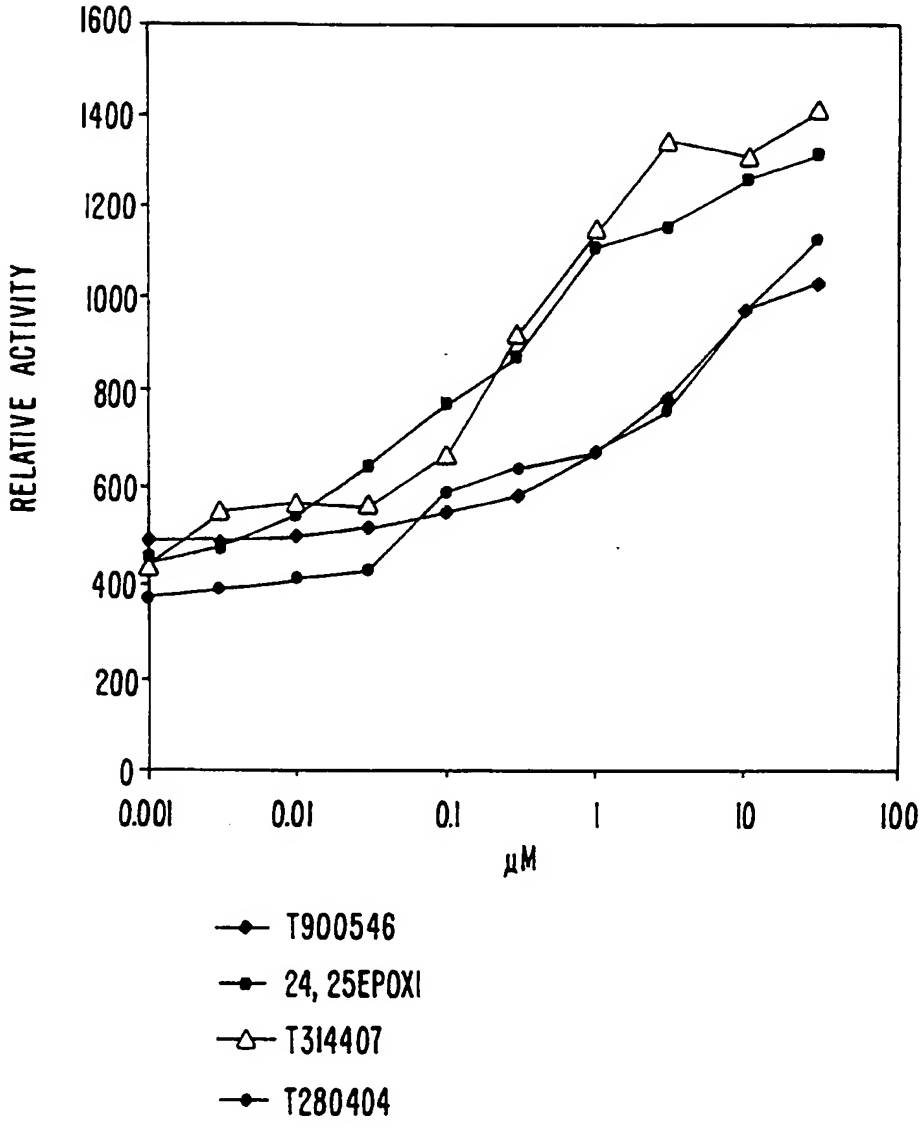

1600
1400
1200
1000
800
600
400
200
0
RELATIVE ACTIVITY
0.001
0.01
0.1
1
10
100
μM
T900546
24, 25EPOXI
T314407
T280404

FIG. 5.

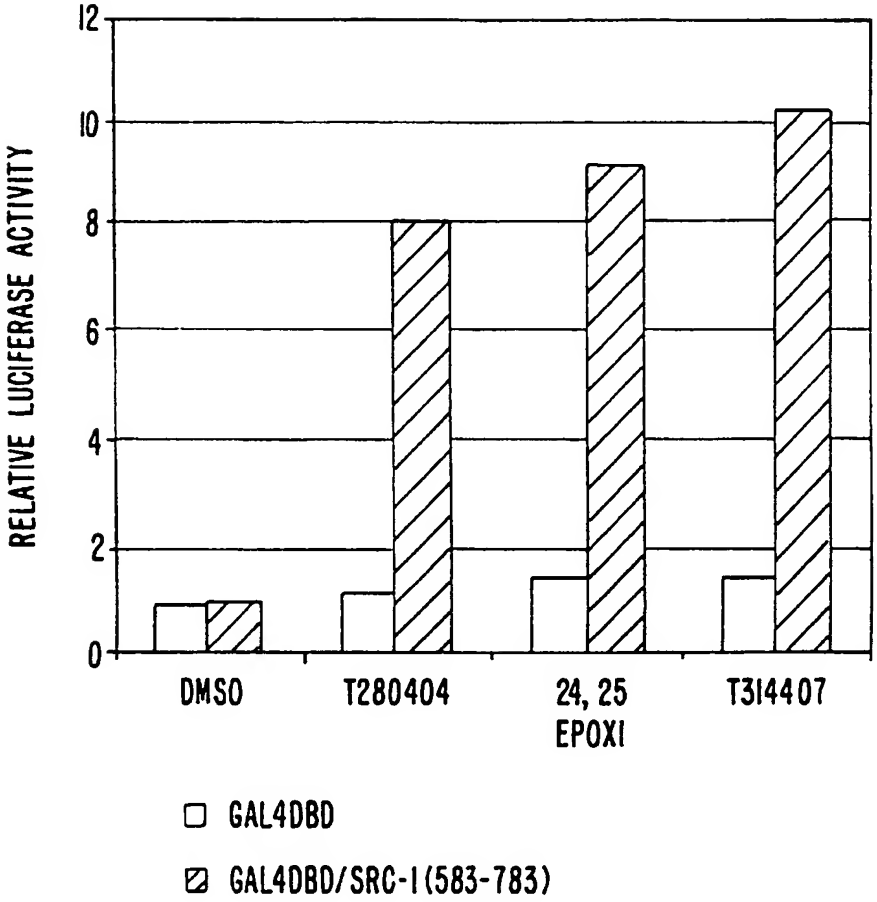

RELATIVE LUCIFERASE ACTIVITY
12
10
8
6
4
2
0
DMSO
T280404
24, 25 EPOXI
T3I4407
GAL4DBD
GAL4DBD/SRC-1(583-783)

FIG. 6.

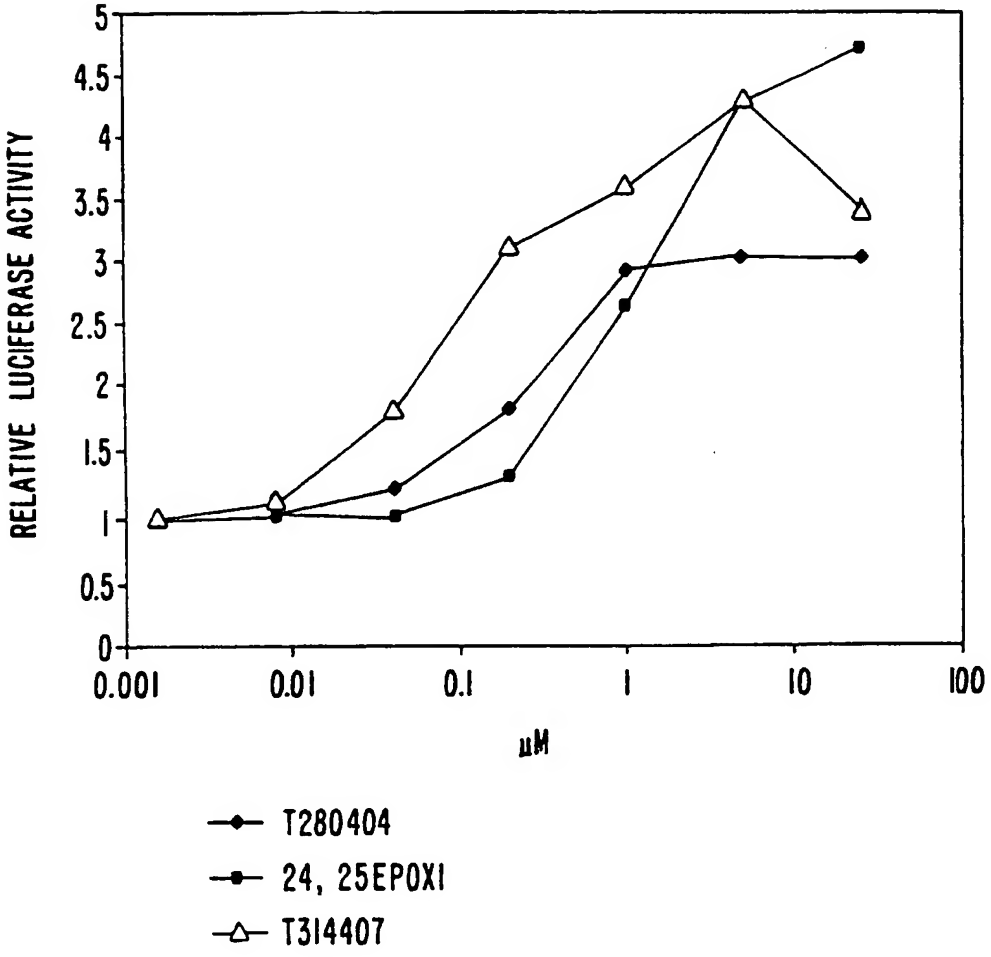

RELATIVE LUCIFERASE ACTIVITY
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0.001
0.01
0.1
1
10
100
µM
T280404
24, 25EPOXI
T314407

FIG. 7.

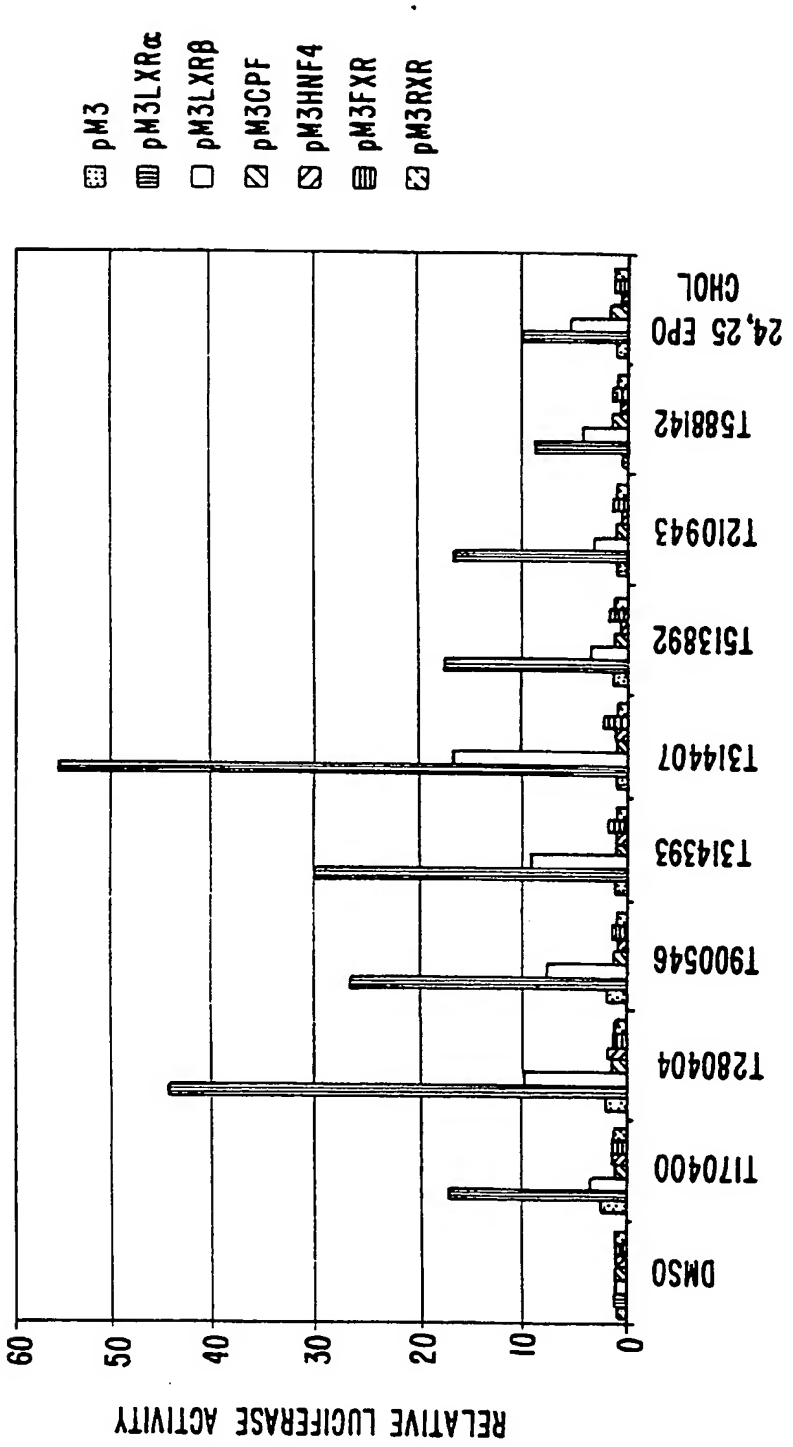

pM3
pM3LXRα
pM3LXRβ
pM3CPF
pM3HNF4
pM3FXR
pM3RXR
RELATIVE LUCIFERASE ACTIVITY
0
10
20
30
40
50
60
DMSO
T1704OO
T280404
T900546
T314393
T31440T
T513892
T210943
T588142
24,25 EPO
CHOL

FIG. 8.

* COMPOUNDS WERE SELECTED FOR PK DETERMINATION BASED ON POTENCY AND STRUCTURAL DIVERSITY.

* COMPOUNDS WERE ADMINISTERED AT 50MG/KG P.O. IN 1% METHYLCELLULOSE SUSPENSION (5MG/ML).

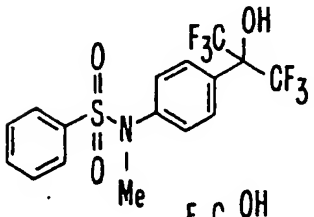
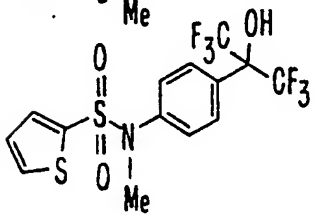
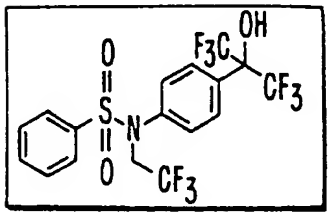
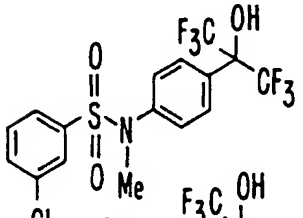
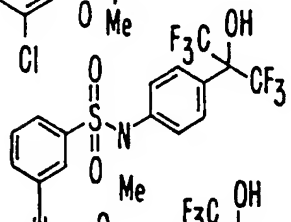
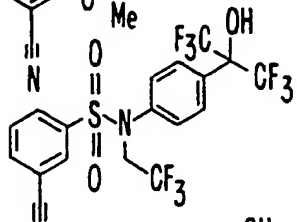
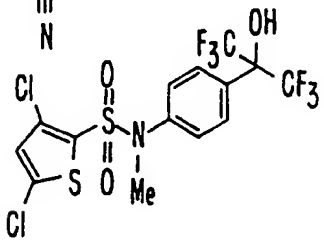
Compound	FP (EC_{50} μM)	FL (EC_{50} μM)	Cytotoxicity (GI50 μM)	Plasma Conc'n @ 1h (μg/ml)
 T0314407	0.3	0.06	>50	8.0
 T0901219	0.5	0.6	>50	17.0
 T0901317	0.05	0.01	30	12.0 5.3 @ 6h
 T0901331	0.05	0.04	50	2.6
 T0901430	0.1	0.02	>50	2.6
 T0901462	0.05	<0.008	>50	0.4
 T0901433	0.02	<0.008	>50	2.9

FIG. 9.

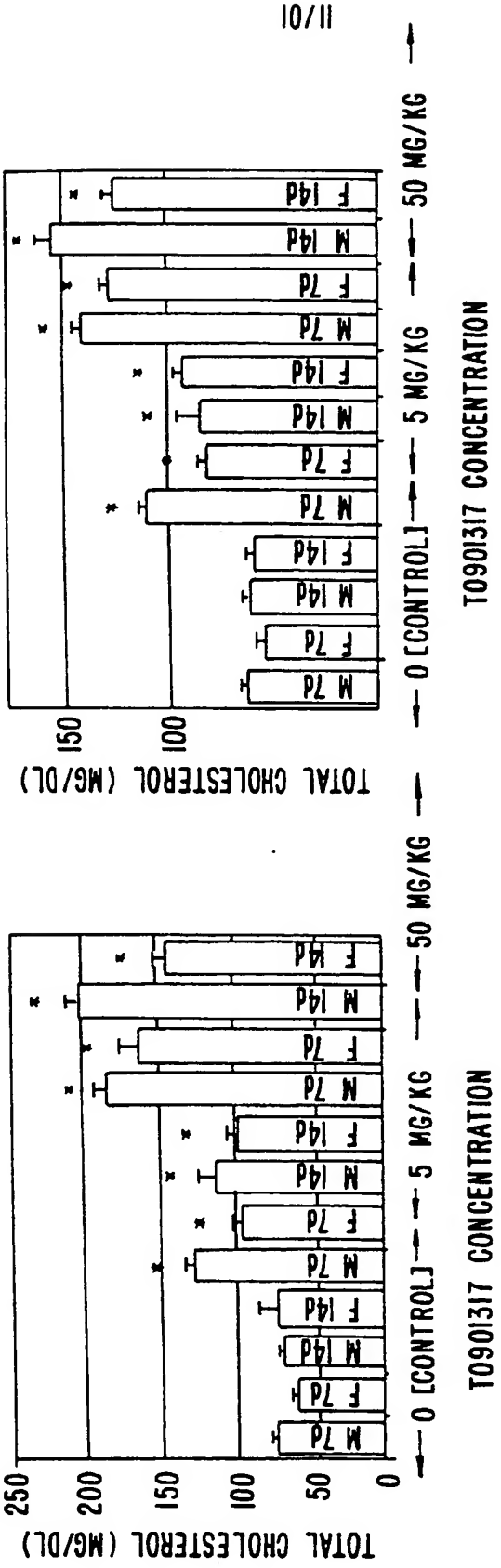

TOTAL CHOLESTEROL (MG/DL)
0
50
100
150
200
250
M 7d
F 7d
M 14d
F 14d
0 [CONTROL]
5 MG/KG
50 MG/KG
T0901317 CONCENTRATION
TOTAL CHOLESTEROL (MG/DL)
100
150
M 7d
F 7d
M 14d
F 14d
0 [CONTROL]
5 MG/KG
50 MG/KG
T0901317 CONCENTRATION

FIG. 10.

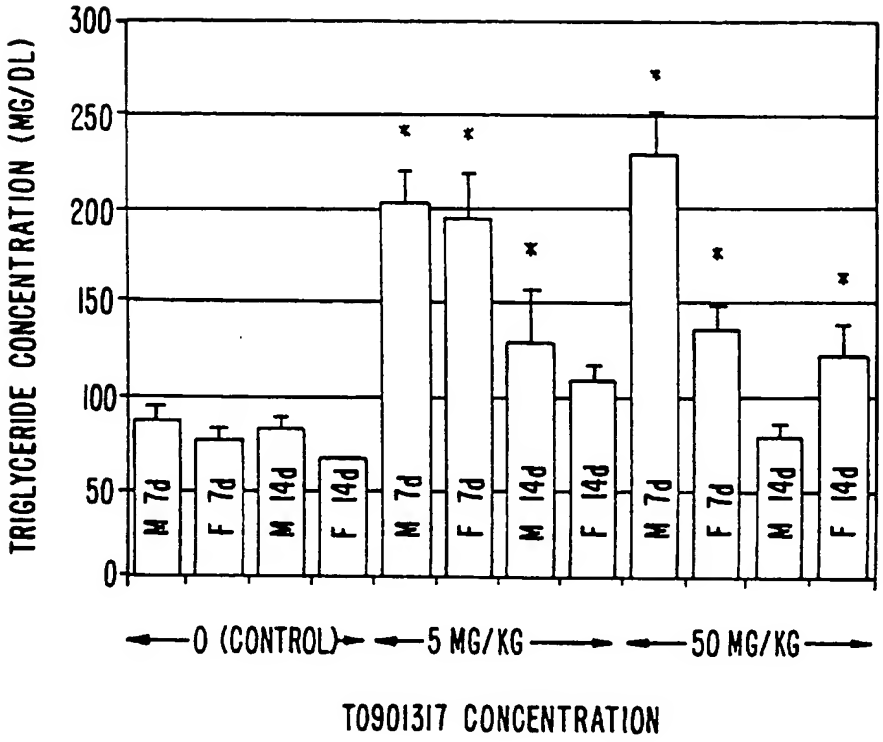

TRIGLYCERIDE CONCENTRATION (MG/DL)
300
250
200
150
100
50
0
M 7d
F 7d
M 14d
F 14d
M 7d
F 7d
M 14d
F 14d
M 7d
F 7d
M 14d
F 14d
0 (CONTROL)
5 MG/KG
50 MG/KG
T0901317 CONCENTRATION

FIG. 11.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/18533

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 31/655, 31/425, 31/42
US CL :514/372, 378

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/156, 372, 378

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS-ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,159,335 A (NEUSTADT) 26 June 1979, see the entire document.	1-43

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 26 SEPTEMBER 2000	Date of mailing of the international search report 18 OCT 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer KEVIN E. WEDDINGTON Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet) (July 1998)*